EFFECTS OF EXTRUDED FLAXSEED AND CONDENSED TANNINS ON RUMEN FERMENTATION, OMASAL FLOW OF NUTRIENTS, MILK COMPOSITION AND MILK FATTY ACID PROFILE IN DAIRY CATTLE

A Thesis Submitted to the College of

Animal and Poultry Science

In Partial Fulfillment of the Requirements

For the Degree of Masters of Science

In the Department of Animal and Poultry Science

University of Saskatchewan

Saskatoon

By

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ABSTRACT

There is interest in increasing the content of omega-3 (n-3; e.g., linolenic acid [C18:3n3]) fatty acids and conjugated linoleic acid (CLA) in bovine milk, primarily because of their beneficial effects on human health. One strategy to alter bovine milk fatty acid composition is the dietary inclusion of flaxseed, which is a rich source of C18:3 n-3. The aim of this study was to evaluate the effects of extrusion processing of flaxseed and the inclusion of condensed tannins (CT) in a flaxseed supplement on omasal flow of nutrients, ruminal fermentation characteristics, animal performance, and milk fatty acid profiles in dairy cattle. Eight multiparous Holstein cows (712.7 ± 92.3 kg body weight; 116.5 ± 17.5 days-in-milk at the beginning of the study) were assigned to four dietary treatments in a replicated 4 x 4 Latin square design consisting of 28-d periods with 20 d of dietary adaptation. Four cows in one Latin square were ruminally-cannulated to allow ruminal and omasal sampling. Cows were fed either a control diet (CTL) or one of 3 treatment diets that consisted of the daily substitution of 3 kg (DM basis) of the CTL concentrate pellet with 3 kg (DM basis) of either a non-extruded flaxseed and pea product (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant; designated RAW), a extruded flaxseed and pea product (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant; designated LPR), or a extruded flaxseed and high-tannin fava bean product (55% flaxseed, 36% high-tannin faba beans, 8% alfalfa, 1% antioxidant; designated LPF). Diets were fed twice daily as total mixed rations. Omasal flow of nutrients was estimated using the omasal sampling technique using iNDF as the single indigestible marker. Dry matter intake was lower ($P = 0.01$) in cows fed the flaxseed diets (24.0 kg/d) compared to those fed CTL (25.9 kg/d). Milk yield was higher ($P = 0.02$) in cows fed the LPR diet (44.4 kg/d) compared to those fed the RAW diet (42.3 kg/d); and tended to be higher ($P = 0.07$) in cows fed the flaxseed diets compared to those fed the CTL diet. Milk fat

yield was unaffected by dietary treatment ($P = 0.94$), whereas milk protein yield tended (P = 0.10) to increase in cows fed the flaxseed diets compared to those fed the CTL diet. No detrimental effects of dietary treatments on ruminal fermentation and omasal flow of microbial protein were observed. The omasal flow of C18:3 n-3 was higher in cows fed the flaxseed diets compared to those fed the CTL diet ($P = 0.04$), with the RAW diet (56.9 g/d) having the highest flow of C18: 3n-3 compared to the other diets (LPR = 14.0 g/d; LPF = 14.8 g/d). The omasal flow of total CLA isomers were higher ($P = 0.03$) in cows fed the LPF diet (6.06 g/d) compared to those fed the LPR diet (3.70 g/d). The C18:3 n-3 content in milk fat (% of fatty acid methyl esters) was higher in cows fed the LPR diet (0.950%) compared to those fed the RAW diet (0.745%). The level of total CLA isomers in milk was also higher in cows fed the LPR diet (0.845%) compared to those fed the RAW diet (0.308%). These results demonstrated that feeding extruded flaxseed products is more effective than feeding whole flaxseed at improving the fatty acid composition of milk fat without negatively impacting animal performance and ruminal fermentation; however, the inclusion of CT in the extruded flaxseed product had no additional benefit.

ACKNOWLEDGMENTS

I acknowledge my co-advisors, Dr. David Christensen and Dr. Tim Mutsvangwa, for their continued support throughout my M. Sc. Program. Additionally I would like to thank the members of my committee including: Dr. John McKinnon, Dr. Peiqiang Yu, and Dr. Shannon Hood-Niefer for their constructive criticisms and invaluable contributions to my research program. Special thanks to Dr. Fiona Buchanan for serving as committee chair.

I gratefully acknowledge O&T Farms Ltd. (Regina, SK.), the National Research Council (Industrial Research Assistance Program) and SaskMilk for providing the necessary funding for this research.

A special thanks to my colleagues in the Animal and Poultry Science Department as well as the staff of the Rayner Dairy Research and Teaching Facility at the University of Saskatchewan for their assistance in sample collections, animal feeding and animal care. I would especially like to thank Breanna Anderson for her tireless commitment and assistance in this trial during her time as our Student Research Associate; I will forever be indebted to you.

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LIST OF ABBREVIATIONS

ADF- Acid detergent fibre

ALA- Alpha-linolenic acid

APE- Atom percentage unit

CLA- Conjugated linoleic acid

CP- Crude protein

Cr- Chromium

CT- Condensed tannins

CTL- Control

CVD- Cardiovascular disease

DHA- Docosahexaenoic acid

DM- Dry matter

DMI- Dry matter intake

DPA- Docosapentaenoic acid

ECM- Energy-corrected milk yield

EE- Ether extract

EPA- Eicosapentaenoic acid

FAB- Fluid associated bacteria

FAME- Fatty acid methyl esters

FP- Fluid particulate phase

HDL- high-density-lipoproteins

iNDF- Indigestible Neutral Detergent Fibre

LA- Linoleic acid

LDL- low-density-lipoproteins

LP- Liquid particulate phase

LPF- linPRO®-F; extruded flaxseed and high-tannin faba bean supplement

LPR- Extruded flaxseed and pea supplement

MFD- Milk fat depression

MUFA- Monounsaturated fatty acids

MUN- Milk urea nitrogen

N- Nitrogen

N3- Omega-3 fatty acids

N6- Omega-6 fatty acids

NANMN- Non-ammonia-non-microbial nitrogen

NAN- Non-ammonia nitrogen

NB- Background nitrogen

NDF- Neutral detergent fibre

NE_g- Net energy gain

NE_l- Net energy of lactation

NE_m- Net energy of maintenance

NH_3-N- Ammonia nitrogen

OM- Organic Matter

OTD- Omasal true digesta

PAB- Particle associated bacteria

PUFA- Polyunsaturated fatty acid

RAW- Non-extruded flaxseed and pea ingredient

SCC- Somatic cell count

SCFA- Short-chained fatty acid

SFA- Saturated fatty acid

SP- Small particulate phase

TMR- Total mixed ration

UFA- Unsaturated fatty acids

WOD- Whole omasal digesta

Yb- Ytterbium

1.0 General Introduction

Interest in altering the fatty acid composition of ruminant products to have elevated levels of polyunsaturated fatty acids (**PUFA**) and conjugated linoleic acids (**CLA**) is growing due to demonstrated human health benefits associated with consumption these fatty acids (Cardoso Carraro et al., 2012; McManus et al., 2011; Simopolous, 2004). Milk is a widely consumed product with a diverse and abundant fatty acid profile with great potential to act as a vector to supply these nutrients to humans.

Inclusion of oilseeds in the diet of dairy cattle is an area of particular interest because of their high PUFA content and relative abundance. Flaxseed (*Linum usitatissium*) is an oilseed that contains a higher concentration of the omega-3 α-linolenic acid (C18:3 n-3) compared to other oilseeds at 55% of total fatty acids (Petit, 2010). However, due to the complex nature of the ruminant gastrointestinal tract, challenges exist in the successful transfer of PUFA from the diet to the milk without negatively influencing rumen metabolism or animal performance. In the rumen, dietary PUFA undergo lipolysis and biohydrogenation by microbial action which alters their structure towards a saturated form (Shorland et al., 1955). Although this process is necessary for the synthesis of CLA, extensive biohydrogeation of dietary PUFA would result in low levels of these fatty acids leaving the rumen to be absorbed within the small intestine and eventually incorporated into the milk (Jenkins et al., 2008).

Several strategies have been explored in an effort to significantly increase the level of PUFA and CLA in bovine milk with limited success in commercial application. Intact oilseeds have been shown to provide partial protection of dietary fatty acids from the ruminal environment (Oba et al., 2009); however, the digestibility of the seeds in the lower gastrointestinal tract may limit the transfer of PUFAs into the milk (Martin et al., 2008, Petit et

al., 2005). Application of heat treatments, such as extrusion, to oilseeds has also been shown to provide partial protection of fatty acids from the ruminal environment through the formation of a protein-matrix surrounding the fat droplets (Kennelly, 1996). Research has shown that the inclusion of extruded flaxseed supplements in dairy cow diets can improve the concentrations of both omega-3 PUFA and CLA in the milk (Neveu et al., 2013; Oeffner et al., 2013). However, the advantages of supplementing dairy diets with extruded flaxseed supplements over intact flaxseed supplements remains unclear. Another potential strategy to increase PUFAs in the milk is through the inclusion of condensed tannins in the cow's diet. Condensed tannins have been shown to limit microbial action against dietary fatty acids (Vasta et al., 2009); therefore, increasing the levels of condensed tannins in an extruded flaxseed product may provide an additive effect over extrusion processing alone in terms of increasing omega-3 PUFA and CLA in the milk.

Although milk fat concentration has been the main area of interest, it is important that all aspects of rumen metabolism and animal performance are also evaluated when supplementing dairy cow diets with flaxseed. Feeding dairy cows diets with high levels of PUFA has been suggested to impair ruminal fibre digestibility (NRC, 2000), milk fat yield (Griinari et al., 1998; Bauman and Griinari, 2003) as well as animal performance (Petit et al., 2015). Impairment of any of these factors could have major economic implications to the producers, especially if producers are being paid a premium for milk fat, as is the case in Canada. Therefore, the aim of this thesis research was to compare the effects of whole flaxseed to extruded flaxseed on omasal flow of nutrients, rumen fermentation, milk composition, milk fatty acid profile, and animal performance in dairy cattle. Furthermore, this study aimed to compare the effects of extruded flaxseed products with varying levels of condensed tannins on the same parameters.

2.0 REVIEW OF LITERATURE

2.1 Bovine Milk Fat and Human Health

Bovine milk fat is one of the most complex forms of naturally occurring lipids and represents a major component of the North American diet. It is estimated that 12% of total dietary fat in Western diets originates from dairy products (Zaripheh and Miller, 2008). Bovine milk fat contains between 3 and 4% total lipids with triacyglycerols representing 95-98% of the lipid fraction and the remainder being composed of cholesterol (0.5%), phospholipids (1%), diacylglycerols (2%) and free fatty acids (<0.5%; Haug et al., 2007). More than 400 individual fatty acids have been identified within these lipid fractions ranging from 4 to 24 carbons in length with varying degrees of saturation and unique biological activities (Haug et al., 2007; Kennelly, 1996; Micinski et al., 2012). The concentration of these fatty acids may vary; Table 2.1 presents the average fatty acid concentrations typically found in Canadian bovine milk (Bilal et al., 2014). The purpose of this section is to review the potential implications of bovine milk fat composition on human health.

2.1.1 Saturated Fatty Acids

Saturated fatty acids (**SFA**) have received a poor reputation over the years due to an assumed link between consumption of SFA and risk of cardiovascular disease (**CVD**). The 2009 American Heart Association Pediatrics and Adult Nutrition Guidelines recommend no more than 7% of total energy intake come from SFA (Gidding et al., 2009). Bovine milk contains high levels of SFA representing 60-70% of total milk fatty acids (Lock et al., 2014). It is estimated that approximately 30% of North American's SFA intake originates from dairy products (Lock et al., 2014). Therefore, there is continued interest related to the effects of bovine milk SFA on human health.

Table 2.1: Average concentration of major fatty acids (g/100g total fatty acids) in the milk of Canadian Holstein cows[1]

Fatty acid carbon number	Mean concentration
C4:0	0.82
C6:0	1.04
C8:0	0.92
C10:0	2.69
C11:0	0.23
C12:0	3.49
C13:0	0.44
C14:0	12.34
C15:0	1.13
C16:0	34.06
C16:1	2.09
C17:0	0.65
C18:0	10.05
C18:1, *trans -9*	0.27
C18:1, *trans-11*	1.29
C18:1, *cis-9*	20.33
C18:2, *cis-9, trans-11*	0.43
C18:2, *trans-10,cis-12*	0.02
C18:2 n-6	1.80
C18:3 n-3	0.40
C20:0	0.12
C20:5n-3	0.03
C22:5n-3	0.10

[1]Adapted from Bilal et al. (2014)

Although milk contains high levels of SFA, few of these fatty acids have been associated with CVD. Lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids have been shown to increase plasma low-density-lipoprotein (**LDL**) cholesterol which has been associated with increased risk for CVD (Denke and Grundy, 1992). However, both C12:0 and C14:0 acids have also been shown to increase high-density-lipoprotein (**HDL**) cholesterol which is considered favorable for human health (Mensink et al., 2003). Other SFA found in milk, such as stearic (C18:0) or those with less than 12 carbons are suggested to have a neutral effect on plasma cholesterol and therefore pose minimal risk to the development of CVD (German and Drillard, 2004).

Due to the complex nature of the SFA fraction of bovine milk, it seems inappropriate to associate all of these fatty acids with increased risk of CVD. A review conducted by German et al. (2009) concluded that there was no consistent epidemiological evidence relating the consumption of dairy products to an increased risk of CVD. Similar results have been reported throughout the literature leading to a general consensus that current dietary recommendations for SFA intake are based on misrepresentation of the data and that the majority of studies fail to support the claim that consumption of bovine milk fat increases the risk of CVD (Calder, 2015; German and Drillard, 2004; Lock et al., 2014; Mensink et al., 2003; Mickinski et al., 2012).

Even though SFA are commonly known for their role in cardiovascular health, it is important to recognize that they have other biological activities that may impact human health. Certain SFA such as C12:0, C14:0 and C16:0 have been associated with insulin resistance and the promotion of inflammation (Calder, 2015). Butyric acid (C4:0) has been recognized for its important role in regulating gene expression (Smith et al., 1998) and prevention of colorectal cancer (Mickinski et al., 2012). Caproic (C6:0) and Caprylic (C8:0) acids have been shown to

have anti-viral activities (German and Drillard, 2004). Additionally, C8:0 has been suggested to have anti-tumor properties which may become applicable for cancer prevention strategies (German and Drillard, 2010).

2.1.2 Unsaturated Fatty Acids

The unsaturated fatty acid (UFA) fraction of bovine milk fat is typically low at 25-30% of total fatty acids (Mickinski et al., 2012). Monounsaturated fatty acids (**MUFA**) consist of a single double bond and represent approximately 83% of the UFA fraction, while polyunsaturated fatty acids (**PUFA**) consist of two or more double bonds and represent approximately 17% of the UFA fraction. The most important MUFA in milk fat, from a quantitative perspective, is oleic acid (*cis-9* C18:1). Human consumption of *cis-9* C18:1 acid has been shown to lower blood pressure, LDL cholesterol, total plasma triacylgylcerides, and improve insulin sensitivity (Kris-Etherton et al., 1999). Additionally, Schwingshackl and Hoffman (2012) conducted a review of 32 studies and found that increased consumption of *cis-9* C18:1 was associated with a reduced risk of CVD and cardiovascular mortality events.

The main PUFA's of interest for human health include the omega-6 fatty acid linoleic acid (C18:2 n-6) and the omega-3 fatty acid α-linolenic acid (C:18:3n-3). Although mammals can synthesize longer-chained PUFAs, they lack both the Δ^{15} and Δ^{12}-desaturase enzymes required for forming *cis* double bonds at the omega-3 and omega-6 positions, respectively (Innis, 2003; Scheffler et al., 1997). Regardless of the absence of enzymes for *de novo* synthesis of C18:2 n-6 and C18:3 n-3, these fatty acids are important for normal physiological and biological functions. For these reasons, both C18:2 n-6 and C18:3 n-3 are classified as essential nutrients for mammalian species (Burr and Burr, 1930; Wesson and Burr, 1931). Even though both nutrients are essential, the biological activities of omega-6 and omega-3 PUFAs are quite

different and their impact on human health should be considered separately. Increasing the concentration of PUFAs in bovine milk is of particular interest since it is typically considered a poor source of PUFA with concentrations of less than 5% of total fatty acids (Vargas-Bello-Pérez and Garnsworthy, 2014).

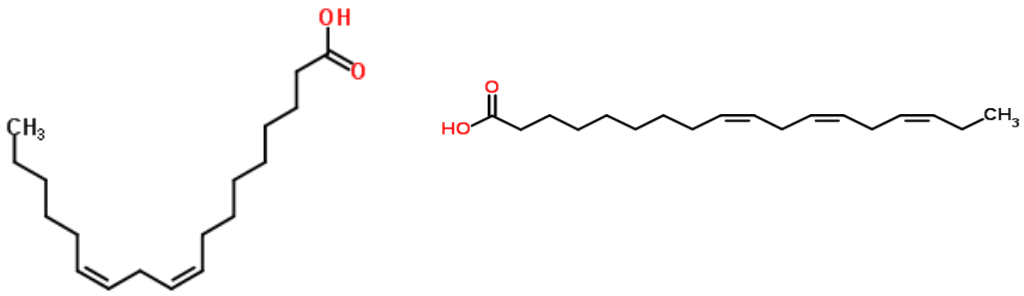
2.1.2.1 Omega-6 fatty acids

Omega-6 fatty acids are PUFA with the first double bond located on the sixth carbon of the fatty acid chain from the methyl end. The parent fatty acid of the omega-6 family is C18:2 n-6 (Figure 2.1). In addition to being an essential nutrient, C18:2 n-6 acts as a precursor for longer chain omega-6 fatty acids such as arachidonic acid (C20:4n-6; Figure 2.2). The ability of 18:2 n-6 to lower blood LDL cholesterol has been well documented (Mensink et al., 2003; Mensink and Katan, 1992); however, its impact on CVD remains uncertain (Calder, 2015). High concentrations of both C18:2 n-6 and C20:4 n-6 are found in membrane phospholipid suggesting that these fatty acids play a crucial role in the maintenance of skin barrier function (Calder, 2015). Omega-6 fatty acids have also been linked to brain development and pro-inflammatory responses (Calder, 2015).

2.1.2.2 Omega-3 fatty acids

Omega-3 fatty acids are PUFAs with the first double bond located on the third carbon of the fatty acid chain from the methyl end. Milk fat content of omega-3 fatty acids is typically low at less than 0.5% total fatty acids with C18:3 n-3 being the primary source (Figure 2.1; Lock et al., 2014). Similarly to C18:2 n-6, C18:3 n-3 is an essential nutrient and a precursor for eicosapentaenoic acid (**EPA**; C20:5 n-3), docosapentaenoic acid (**DPA**; C22:5 n-3) and docosahexaenoic acid (**DHA**; C22:6 n-3) as illustrated in Figure 2.2 .There is particular interest in the role these fatty acids play in cardiovascular health, inflammation, neurological

development and cancer prevention. The rate at which the long-chain PUFAs are synthesized is dependent on the level of enzymatic efficiency at each step of elongation (Alhazzaa et al., 2013). According to a study by Alhazzaa et al. (2013), the biosynthesis of EPA and DPA had the slowest enzymatic efficiencies suggesting the activities of Δ^5-desaturase and elongase-2 may be the rate limiting steps in biosynthesis of DHA.



(A) **(B)**

Figure 2.1: Chemical structures of linoleic acid (A) and α-linolenic acid (B) (National Center for Biotechnology Information. 2015)

The biological roles of C18:3 n-3 besides being a precursor for longer chain omega-3 fatty acid is sometimes overlooked; nevertheless, C18:3 n-3 plays an important role in mediating inflammatory responses and neurological health. A study conducted by Ferruci et al. (2006) found that higher levels of plasma C18:3 n-3 was associated with an increase in anti-inflammatory biomarkers. Based on these and similar findings, it is suggested that increased consumption of C18:3 n-3 has the potential to protect against inflammatory diseases such as rheumatoid arthritis, Crohn's disease, irritable bowel syndrome and heart disease (Stark et al., 2008). Additionally, C18:3 n-3 has been suggested to play an important role in prevention of

autoimmune diseases (Reiffen et al., 1998) as well as neurological illnesses such as attention deficit hyperactivity disorder (Joshi et al., 2006).

The fatty acids C20:5 n-3 and C22:6 n-3 have both been shown to play an important role in neurological development, cardiovascular health and cancer prevention (Lock et al., 2014). It is believed that C22:6 n-3 plays a large role in brain and visual development and may also be important for the prevention of depression and schizophrenia (Calder, 2015). Additionally, both C20:5 n-3 and C22:6 n-3 have demonstrated anti-inflammatory activities (Calder 2015), and an ability to lower blood triacylglycerols (Shearer et al., 2012) which may be important in the prevention of CVD. Some studies also investigated the effects of C20:5 n-3 and C22:6 n-3 on the risk of breast, prostate and colorectal cancers (Gerber, 2012; Makarem et al., 2013); however, the results are inconsistent and more work needs to be done in this area.

2.1.2.3 Omega-6/Omega-3 ratio

It is accepted that both C18:2 n-6 and C18:3 n-3 are essential nutrients and exhibit unique biological activities; however, both elongation pathways require the same enzymes suggesting competition exists between C18:2 n-6 and C18:3 n-3 for the rate limiting Δ^6-desaturase enzyme (Simopoulos 2002; Figure 2.2). It is suggested that increased consumption of one PUFA family compared to another may cause an imbalance in inflammatory responses in the body and lead to increased risk of chronic diseases (Simopoulos, 2006). According to Simopoulos (2006), humans historically consumed diets with an omega-6 to omega-3 ratio of 1:1 compared to modern dietary patterns consisting of a 20:1 ratio. The increased consumption of omega-6 fatty acids in Western diets is believed to limit the elongation of C18:3 n-3 and potentially limit the ability of omega-3 fatty acids to mitigate inflammation (Simopoulos, 2006). Increased consumption of omega-6 fatty acids, therefore, has been suggested to be responsible for the increased incidences of

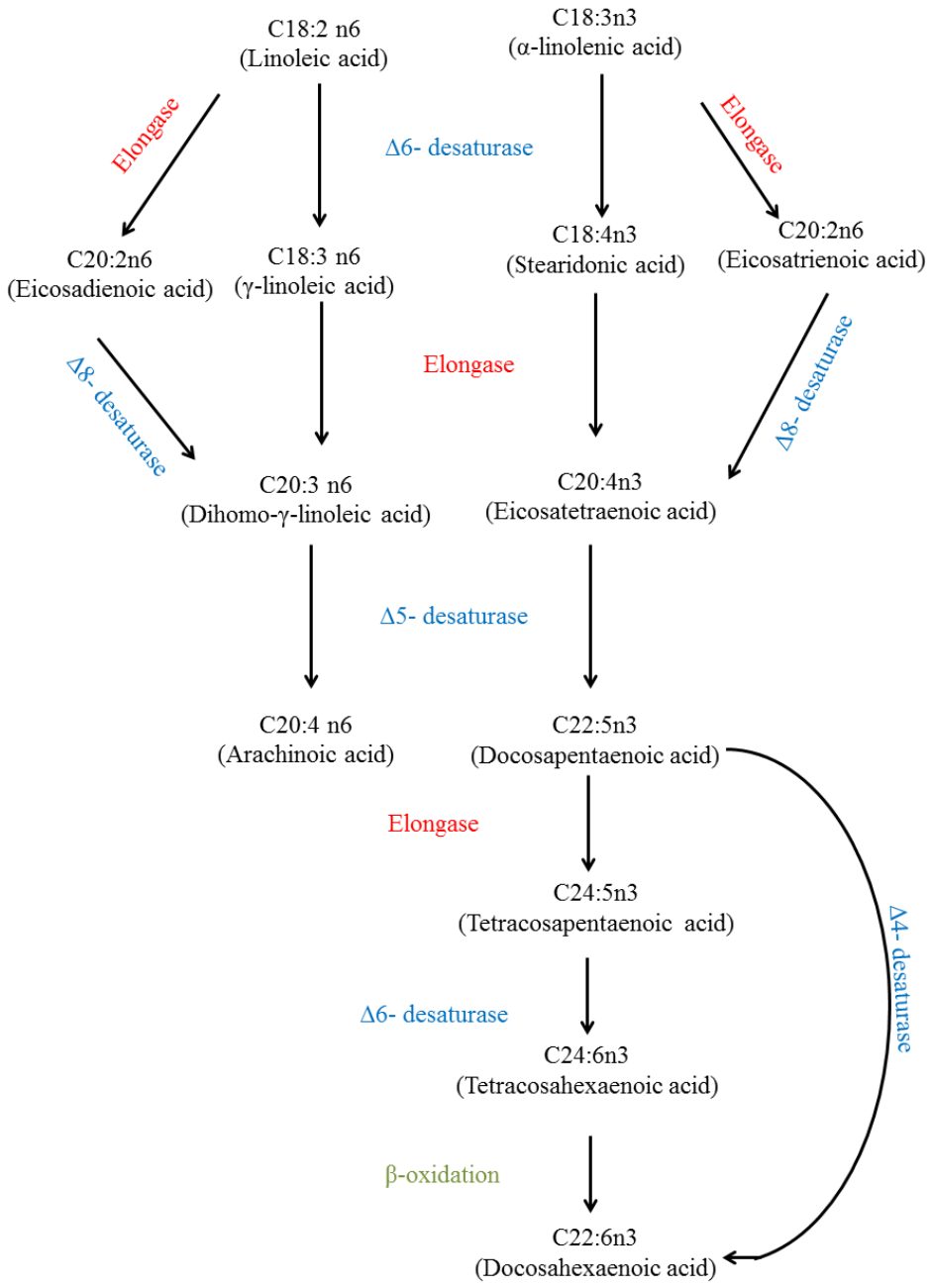


Figure 2.2: Biosynthesis of polyunsaturated fatty acids from Linoleic acid and alpha-linoleic acid (Adapted from Chuang et al., 2001; Chilton, 2014; Grundt et al., 2014).

inflammatory illnesses such as type 2 diabetes, cancer and obesity currently exhibited in Western society (Cardoso Carraro et al., 2012). However, there is limited epidemiological evidence for these claims and more clinical trials are necessary to support this theory.

2.1.3 Trans fatty acids

Milk fat is the richest natural source of CLA fatty acids (Kraft et al., 2003). The term CLA refers to a group of octadecadienoic acid isomers synthesized through the incomplete biohydrogenation of linoleic acid in the rumen, or through desaturase activities in the mammary gland of ruminant animals. The CLA fatty acids were first identified as a mutagen inhibitor by Ha et al. (1987) which led to extensive research of these fatty acids as anti-carcinogens. Research has found that certain CLA isomers are able to suppress the growth of cancer-cells involved in breast cancer, skin cancer, prostate cancer and stomach cancer (Lee et al., 2005). Although bovine milk fat is considered the richest source of these anti-carcinogen agents, the concentration of CLA isomers in bovine milk is highly variable. More research is required to better understand the impact of external and physiological factors on CLA enrichment of milk.

2.1.4 *The Ideal Milk Fat*

The nutritional value of bovine milk fat is well documented; however, the potential to alter individual fatty acids within the lipid fraction continues to be of interest for human health. Although many SFA present in the milk have a neutral or beneficial impact to human health, others such as C12:0, C14:0, and C16:0 remain a concern. Developing strategies to replace some of these SFA with more beneficial MUFA or PUFA has the potential to improve the fatty acid profile of bovine milk and contribute to improved human health. A meta-analysis conducted by Mensink et al. (2003) reported reduced risk of CVD when SFA were replaced with MUFA or PUFA. The literature also suggests that increasing the availability of omega-3 PUFAs appears to

be of more relevance compared to omega-6 fatty acids based on current dietary intakes in North America (Simopolous, 2006). Furthermore, increasing the concentrations of CLA isomers in bovine milk fat may provide additional nutritional benefits for consumers.

2.2 Ruminal Lipid Metabolism

The fatty acid composition of digesta and microorganisms leaving the rumen and entering the small intestine can have a major influence on the fatty acid composition of the milk. These fatty acids can be absorbed in the small intestine, enter the circulatory system and become available for milk fat synthesis which will be discussed in later sections. The fatty acid profiles of the diet fed to a ruminant animal and the digesta entering the lower gastrointestinal tract are different, thus making it difficult to predict milk fat composition based on the diet. This variation can be explained by the activities of the microbial ecosystem within the rumen that consists of approximately 10^{10} bacteria, 10^{7} protozoa and 10^{6} fungi per ml of ruminal fluid (Buccioni et al., 2012).

Microbial fermentation of carbohydrates results in the production of short-chained fatty acids (**SCFA**) as a byproduct. Furthermore, certain microbial species are able to saturate dietary unsaturated fatty acids through a process known as biohydrogenation (**BH**). These activities greatly influence the flow of fatty acids to the small intestine and subsequently, the fatty acid composition of the milk. As such, understanding ruminal lipid metabolism is a crucial step towards the development of dietary strategies designed to improve milk fat composition.

2.2.1 Production of short-chain fatty acids in the rumen

The microbial population within the rumen is effective at fermenting dietary carbohydrate and protein sources to yield SCFA. The most important SCFAs produced- from a quantitative perspective- include acetate, propionate and butyrate. Acetate and butyrate play an

important role in *de novo* milk fatty acid synthesis (Palmquist, 2006). As such, the proportion in which SCFAs are produced in the rumen may have a significant impact on the fatty acid profile of bovine milk.

The production of acetate and butyrate are positively linked to milk fat production. Butyrate synthesis is relatively constant with few means of increasing its concentrations (Chillard et al., 2000). Acetate is primarily produced from fibrolytic bacteria and, therefore, the presence of slowly-degradable fibre sources is important for its production. Diets that may impair or limit fibre digestion such as high grain diets or diets with unprotected unsaturated fatty acids may subsequently limit acetate production and synthesis of milk fatty acids (Chillard et al., 2000).

The role of propionate in milk fat synthesis is often considered to be minimal; however, it is still important to evaluate the role of all major SCFAs on milk fat synthesis and composition. Even though it may not have a direct impact on the production of fatty acids, propionate is the primary precursor for gluconeogenesis in ruminant species. As such, propionate can influence the availability of glycerol for TAG synthesis in the mammary gland. The proportion of propionate relative to acetate and butyrate must also be considered. Increased concentrations of propionate may limit the flux of acetate and butyrate, thereby, limiting substrate availability for *de novo* milk fat synthesis (Chillard et al., 2000). Increasing the concentrate proportion of the diet, especially with rapidly fermentable carbohydrates will favor the production of propionate. Other dietary factors such as the presence of ionophores and intake of long-chained fatty acids may also increase propionate synthesis (Chillard et al., 2000).

2.2.2 Dietary lipid metabolism in the rumen

The structures of dietary fats are altered by rumen microorganisms through the processes of lipolysis and biohydrogenation. The combined result of these processes is reduced rumen outflow of UFA and an accumulation of CLA isomers and biohydrogenation intermediates (Figure 2.3).

Lipolysis results in the release of free fatty acids (**FFA**) from their glycerol backbone (Buccioni et al., 2012). Upon ingestion of esterified dietary lipids, microbial lipases carry out this process and then utilize the glycerol for the synthesis of the SCFA propionate (Garton et al.

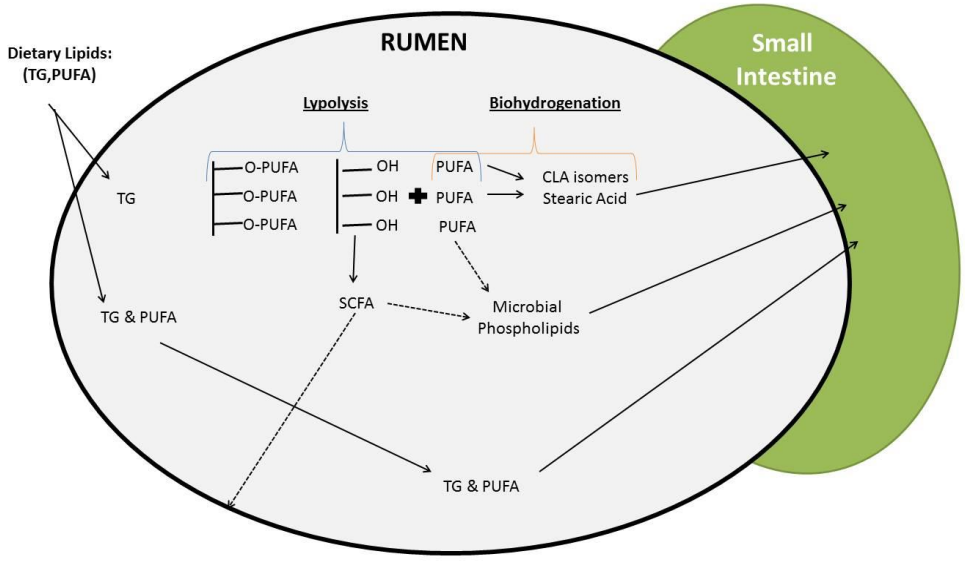


Figure 2.3: Schematic of rumen metabolism of dietary PUFA (TG = triglycerides, PUFA= polyunsaturated fatty acids, CLA=Conjugated linoleic acid, SCFA=short chain fatty acids). Modified from Lock et al. (2006).

1960). The rate at which lipolysis is carried out varies based on the type of dietary lipids being ingested by the animal and the type of microorganisms present in the rumen. For example, *B. fibrisolvens* and *A. lipolytica* are recognized for their lipolytic activities; however, the lipase

activity of these microorganisms is highly specific with varying rates of hydrolysis (Fay et al., 1990). For example, both *B. fibrisolvens* and *A. lipolytica* can hydrolyze ester bonds; however, *B. fibrisolvens* is capable of hydrolyzing phospholipids while *A. lipolytica* hydrolyzes di- and triglycerides (Buccioni et al., 2012). Galactolipase and phospholipase activities are also active in other microbial species for the hydrolysis of galactolipids and phospholipids, respectively (Jenkins 1993).

Biohydrogenation is the next step in rumen metabolism of UFA. The ability of rumen microorganisms to hydrogenate UFA was first demonstrated by Reiser (1951) by incubating flaxseed oil in the rumen contents of sheep. Shortly after, Shorland et al. (1955) demonstrated the conversion of C18:3 n-3 to more saturated derivatives when the fatty acid was incubated in rumen contents. The process of biohydrogenation is believed to consist of two steps, which are: 1) isomerization and 2) reduction. The isomerization of UFA ensures the fatty acid is in the appropriate configuration to allow hydrogenation by converting a *cis* configuration into a more stable *trans* configuration. For example, Kepler and Trove (1967) demonstrated the ability of *Butyrivibrio fibrisolven* species to change the configuration of linoleic acid from *cis*-12 to *trans*-*11* via the linoleate isomerase enzyme. Once isomerization is complete, Rosenfeld and Trove (1971) demonstrated *B. fibrisolven'*s ability to hydrogenate the fatty acid chain resulting in the formation of a *trans*-*11* C18:1 fatty acid from the *cis*-*9, trans*-11 CLA (Figure 2.4). The potential sources of electron donors for the hydrogenation process may include H_2O, NADH, methyl violegen, and endogenous sources such as α-tocopherolquinol (Hunter et al., 1976; Hughes and Tove, 1980).

The discovery of rumen microbial species that were able to hydrogenate UFA raised several questions regarding the purpose of this processes. The most widely accepted explanation

for these activities relates to the impact UFA have on microbial growth. The sensitivity and growth of *Butyrivibrio* bacterial species in the presence of different fatty acids was investigated by Maia et al. (2006) and reported a decrease in growth of *Butyrivibrio fibrisolven* in the presence of C18:3 n-3 and CLA isomers. However, *tran-11* C18:1 fatty acids had no effect on the growth of this species. Based on these findings, it was concluded that UFA exhibit a toxic effect on certain microbial species and impair their growth; therefore, hydrogenation of UFA reverses these inhibitions (Jenkins, 2008).

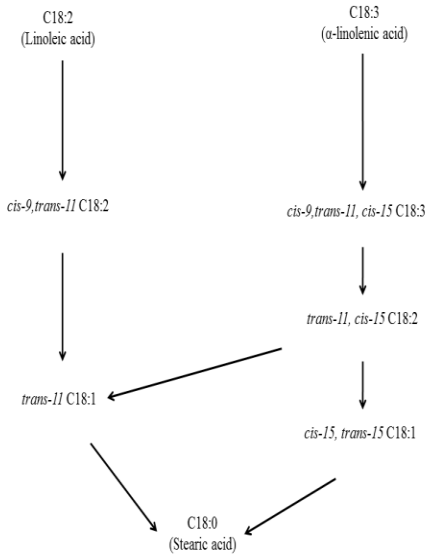


Figure 2.4: Biohydrogenation pathway of linoleic acid to stearic acid in the rumen. Adapted from Harfoot and Hazelwood (1997)

2.3 Mammary Lipid Metabolism

Successful manipulation of bovine milk fat composition requires a thorough understanding of its origin. Bovine milk fatty acids originate from two primary sources which are *de novo* synthesis and uptake of circulating fatty acids by the mammary gland (Bauman and Griinari, 2003). The short-chain (4 to 8 carbons) and medium-chain (10-14 carbons) fatty acids

found in milk originate exclusively from *de novo* synthesis while long-chain fatty acids (greater than 16 carbons) originate from mammary uptake of circulating fatty acids. The C16:0 fatty acids are an exception in that they can originate from either source.

2.3.1 De novo fatty acid synthesis in mammary gland

De novo fatty acid synthesis accounts for approximately 40% of total milk fatty acids (Chillard et al., 2000) as is illustrated in Figure 2.5. The process of fatty acid synthesis requires both carbon sources and reducing equivalents. In ruminants, acetate and β-hydroxibutyrate are considered the primary carbon sources, while $NADPH+H^+$ represents the primary reducing equivalent and originates from either the oxidation of glucose or the oxidation of isocitrate (Palmquist, 2006). Acetic acid is converted to acetyl-CoA by the mammary epithelial cells which is then used for the synthesis of malonlyl-coA via acetyl-coA carboxylase. The conversion of acetyl-CoA to malonyl-CoA is believed to be the rate limiting step of fatty acid synthesis (Shingfield et al., 2010). The activity of acetyl-CoA carboxylase can be down-regulated in the presence of long-chain fatty acids and, therefore, decreased synthesis of short- and medium-chain milk fatty acids (Shingfield et al., 2010). Fatty acid synthetase is the enzyme responsible for fatty acid synthesis from malonly-CoA with butyryl-CoA and acetyl-coA acting as carbon sources for chain elongation (Cozma et al., 2013).

In addition to fatty acid synthesis, the mammary gland has the capacity to alter fatty acid structure through the activities of the Δ^9-desaturase enzyme. The Δ^9-desaturase enzyme is located in the mammary secretory cells and can add a *cis*-9 double bond to long-chain fatty acids (Shingfield et al., 2008). The desaturation of C18:0 to *cis-9* C18:1 in the mammary gland is suggested to be responsible for 80% of the *cis-9* C18:1 found in bovine milk (Shingfield et al., 2013). Similarly, *trans-11* C18:1 can be desaturated to the CLA isomer *cis-9, trans-11* C18:2

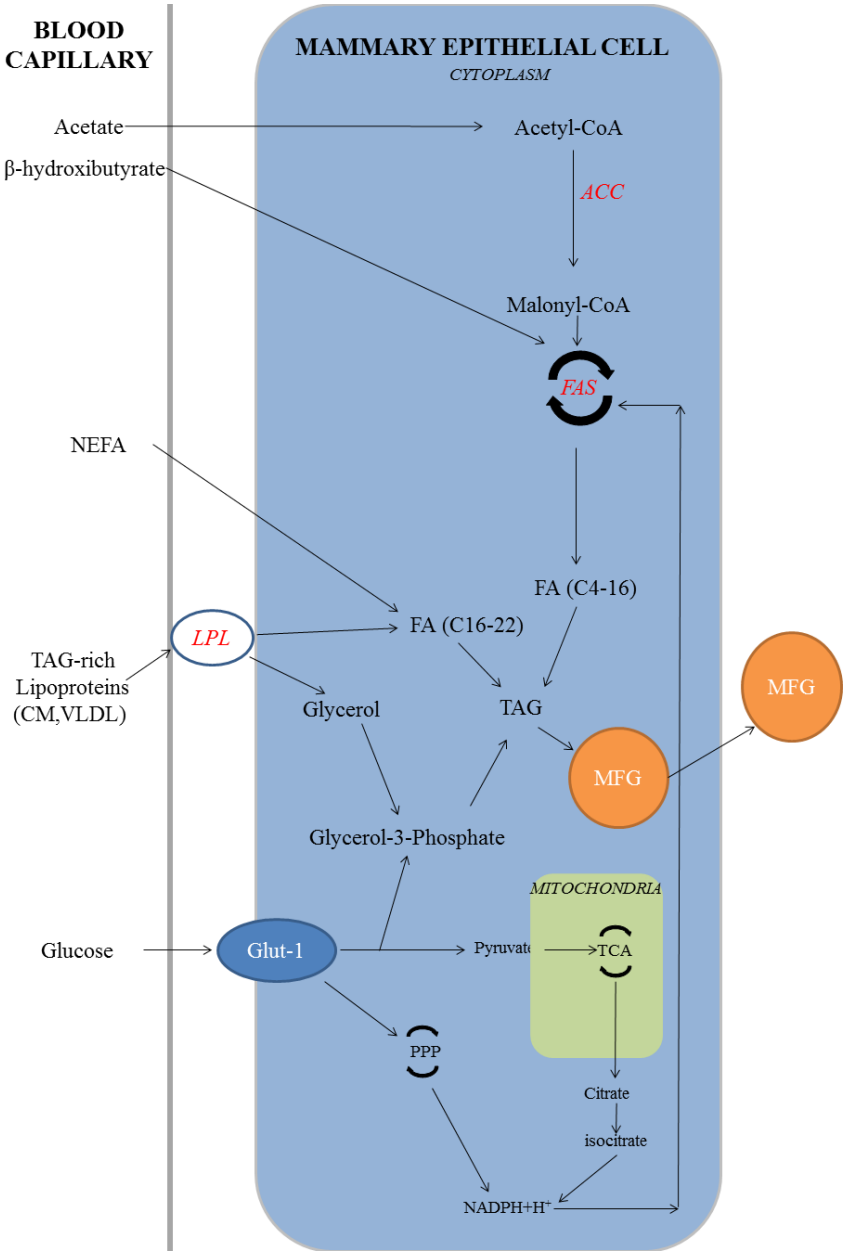


Figure 2.5: Biosynthesis of milk fat. Abbreviations: ACC= Acetyl-CoA Carboxylase, FAS= Fatty Acid Synthetase, TAG= triacylgliceride, CM= chilomycron, VLDL= very-low-density-lipoprotein, LPL=lipoprotein lipase, FA= fatty acid, PPP=pentose phosphate pathway TCA=tricarboxylic acid cycle, MFG= milk fat globule, NEFA= non-esterfied fatty acids. (Adapted from Palmquist, 2006; Shigfield et al., 2010; and Chillard et al., 2000)

and this *de novo* synthesis accounts for 70-95% of cis-*9, trans-11* C18:2 recovered in the milk (Shingfield et al., 2013). Other fatty acids such as C10:0, C12:0, C14:0, C15:0 and C17:0 may also be desaturated through this pathway (Shingfield et al., 2010).

2.3.2 Uptake of circulating fatty acids by mammary gland

Fatty acids taken up by the mammary gland originate primarily from the intestinal absorption of dietary and microbial lipid in the form of non-esterified fatty acids (**NEFA**) or triglyceride-rich lipoproteins such as chylomicrons or very-low-density lipoproteins (Bauman and Griiani 2003; Figure 2.5). The ability of the mammary gland to utilize triglyceride-rich lipoproteins is dependent on the activity of the enzyme lipoprotein lipase to transfer these lipids into the mammary secretory cell (Chillard et al., 2010). A smaller portion of circulating lipids (<10%) taken up by the mammary gland originates from the mobilization of adipose tissues (Cozema et al., 2013). The contribution of adipose tissue may vary and is more common in animals experiencing negative energy balance (Palmquist and Jenkins, 1980). The adipose tissue contains high levels of both C18:0 and *cis-9* C18:1; therefore, when adipose tissue is being mobilized by the animal an increase in C18:0 and *cis-9* C18:1 is observed in the milk (Chillard et al., 2003).

2.3.3 Packaging of fatty acids in the mammary gland

The fatty acids that are synthesized *de novo* or taken up from circulation can then be esterified to form triacylglycerols. The esterification of fatty acids to the glycerol backbone is highly specific forming asymmetrical arrangements of the triacylglycerol molecules. The fatty acids C4:0, C6:0 and cis-*9* C18:1 are preferentially esterified at the sn-3 position (Jensen, 2002), while the fatty acids C8:0, C10:0, C12:0, and C14:0 are esterified at the sn-2 position (Cozema et al., 2013). The distribution of C16:0 is equal between the sn-1 and sn-2 position and C18:0 is

esterified at the sn-1 position (Cozma et al., 2013). It is believed that the positioning of *cis-9* C18:1 plays an important role in maintaining the physical properties of milk by maintaining milk fat melting point similar to that of the cow's body temperature for efficient utilization by the calf (Timmen and Pattron, 1988). The preferential packaging of the *cis-9* C18:1 fatty acids, especially, further emphasizes the functional role of desaturase activity within the mammary secretory cells, as was previously discussed.

2.3.4 Milk fat depression

A correlation between milk fat synthesis and animal diet was first reported by Powell (1939). Since then, several theories have been deduced in an attempt to explain diet-induced milk fat depression (**MFD**). One theory suggests changes in rumen fermentation that limit acetate and butyrate production will inhibit milk fat synthesis (Doreau and Chillard, 1999). Another theory suggests that an increase in insulin production and repartitioning of fat towards adipose tissue, commonly known as the "glucose-insulin theory", causes MFD (McClymont and Vallance, 1962). However, the most widely accepted theory to-date is the "biohydrogenation theory" proposed by Bauman and Griinari (2003).

The "biohydrogenation theory" suggests that certain dietary conditions can alter the biohydrogenation pathway of dietary unsaturated fats resulting in an accumulation of intermediates that inhibit milk synthesis in the mammary gland (Bauman and Griinari, 2003). In order for production of these intermediates to occur, two conditions must be met: 1) that there is a supply of dietary UFA; and 2) that microbial activity within the ruminal ecosystem shifts towards an alternative biohydrogenation pathway (Griinari et al., 1998). The specific changes in the rumen environment to elicit such a response in the microorganism include a low-forage diet associated with a lower ruminal pH (Figure 2.6; Peterson et al., 2002a).

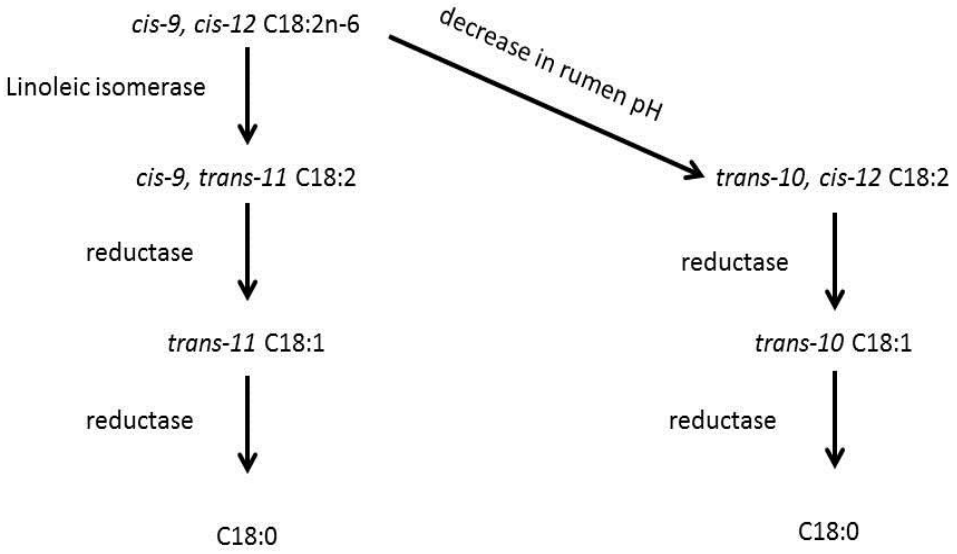


Figure 2.6: Shift in biohydrogenation pathway of linoleic acid with lower ruminal pH (Adapted from Griinari and Bauman 1999).

The theory proposed by Bauman and Griinari (2003) suggested that the *trans-10* C18:1 intermediate was to blame for MFD. However, there was little evidentiary support directly linking this fatty acid to milk fat synthesis in the mammary gland. Instead, research has demonstrated that the *trans-10, cis-12* CLA isomer is more closely associated with diet-induced MFD. Infusion of *trans-10, cis-12* CLA into the abomasum of dairy cattle has resulted in a decrease in milk fat yield as high as 25% (Peterson et al., 2002b). The mechanism by which the *trans-10, cis-12* CLA isomer inhibits milk fat synthesis appears to be through the down-regulation of genes encoding for mammary lipogenic enzymes. Piperova et al. (2000) observed a 40-60% reduction in mRNA encoding for the acetyl coenzyme A (acetyl-CoA). This observation was accompanied by a reduction in milk fat yield in animals offered a low-forage diet. Similar results have been reported by Baumgard et al. (2002) who observed a decrease in mRNA genes

encoded for acetyl-CoA, fatty acid synthetase, Δ^9-desaturase as well as lipoprotein lipase in mammary tissue of animals treated with *trans-10, cis-9* CLA.

2.4 Dietary Strategies to Alter Bovine Milk Fat

The composition and quality of bovine milk fat can be influenced by several factors such as: stage of lactation, seasonal variations, genetics, and nutrition (Ashes et al., 1997). Nutritional management is the most rapid and effective strategy to elicit a response in milk fat composition. Supplementing the animal's diet with a fat source, in particular, can have a substantial impact on milk fat yield and fatty acid composition. The purpose of this section is to review the impact of dietary fat supplementation on milk composition with particular emphasis on the use of flaxseed in dairy rations. It is important to note that the effectiveness of fat supplements may be influenced by interactions with other dietary components. For this reason, a brief overview of condensed tannins and forage-to-concentrate ratio will also be mentioned.

2.4.1 Fat Supplementation

The supplementation of dairy rations with fat has been extensively researched over the past 40 years. In the 1980s, the primary use of fat supplements was to improve the energy density of the diet and to promote milk production (Jenkins and McGuire, 2006). Initial research investigating the effects of including fat in dairy rations typically included information regarding the fatty acid composition of the milk (Jenkins and McGuire, 2006). The data from these initial trials, combined with a growing interest of the effect of fatty acids on human health, contributed to our current understanding of the impact fat supplementation had on milk fat composition and quality.

Fat supplements come in a wide range of forms, sources, and compositions. Some of the most common types of fat supplements used in dairy rations include vegetable sources, tallow,

oilseeds, and marine sources. According to Ashes et al. (1997), there are several features of a fat supplement that must be considered, such as: the type of fat, the fatty acid composition, the degree of protection from biohydrogenating microflora within the rumen, transfer efficiency of fatty acids into the milk, and impact on *de novo* milk fat synthesis.

There is particular emphasis on increasing the concentration of PUFAs in bovine milk and decreasing the concentration of SFA to better accommodate consumer demand. Although fat supplements high in PUFA present significant opportunity for product development and human health, there are several challenges that must be overcome. The main challenge relates to biohydrogenation processes that occur within the rumen, as was described earlier. Jenkins and Bridges (2007) conducted a meta-analysis to evaluate the duodenal flow of fatty acids from animals fed different fat supplements. The results of this study found that the flow of unsaturated fatty acids between a control diet with no fat supplementation, and the flow of a diet supplemented with an unprotected source of PUFAs did not differ. Jenkins and Bridges (2007) estimated an 83% loss of dietary C18:2 n-6 and an 87% loss of dietary C18:3 n-3 through biohydrogenation processes. Since the fatty acids flowing from the rumen are closely linked to the fatty acids recovered in the milk, these findings emphasize the importance of rumen protection when using fat supplements to alter milk fatty acid composition.

2.4.1.1 Protection of Fat Supplements

The inclusion of fat in the diet of dairy cattle increases the risk of impaired rumen digestion as well as a loss of dietary unsaturated fatty acids through biohydrogenation. To mitigate these issues, researchers have evaluated different strategies to protect dietary fatty acids from the biohydrogenating microflora in the rumen and increase their availability in the small intestine. According to Jenkins and Bridges (2007), the criteria required for the success of

rumen protection technologies include: 1) consistent and predictable enhancement of unsaturated fatty acid flow at the duodenum, 2) adequate release and absorption of fatty acids in the small intestine, and 3) minimal impact on ruminal fermentation.

In the early 1970's, a research team from Australia was the first to develop an effective process for protecting dietary UFA from biohydrogenation. Sunflower oil was encased in a protein-shell and treated with formaldehyde to form cross-linkages that were resistant to microbial degradation (Cook et al., 1972; Pan et al., 1972). Gooridge et al. (2001) reported a 67% and 42% increase in the concentrations of milk C18:3 n-3 and C18:2 n-6, respectively, when formaldehyde-treated flaxseed was offered to lactating dairy cattle. Although effective, the application of formaldehyde-treated oilseeds in a commercial diet remains controversial. While earlier studies have negated any potential health risks associated with feeding formaldehyde-treated products to cattle (Mills et al., 1972; McDonald and Scott, 1977), there are still concerns regarding potential negative impact on intestinal digestibility and potential health risks associated the products (Mir et al., 1984; Jenkins and McGuire, 2006).

An alternative strategy to formaldehyde-treated products could be the use of calcium salts of fatty acids. The application of calcium salts in dairy diets was developed at The Ohio State University where calcium salts of palm oil showed resistance to rumen biohydrogenation (Jenkins 2006). Similarly, Lundy et al. (2004) reported enhanced omasal flow of UFA when soybean oil with calcium salts was fed to dairy cattle. Although calcium salts of fatty acids have been shown to resist rumen biohydrogenation compared to free oils, they do not provide a high level of rumen protection and therefore could result in milk fat depression due to the production of biohydrogenation intermediates (Harvatine, 2015).

Heat processing methods such extrusion and micronization have been shown to provide partial protection of dietary UFA from the microflora within the rumen capable of biohydrogenation. Kennelley (1996) suggests that the application of heat to high oil products such as oilseeds may protect unsaturated fatty acids from microbial biohydrogenation in the rumen by forming a protective protein matrix surrounding the fat droplets. Dry extrusion uses friction as its sole source of heat and has been reported to reach temperatures between 120°C and 155°C (Chouinard et al., 2001; Gonthier et al., 2005). Similarly, micronization is a dry heat treatment that uses infrared gas generators to reach temperatures of 110°C and 115°C (Petit, 2010). Doreau et al. (2009) reported increased duodenal flow of C18:3 n-3 when comparing extruded flaxseed and rolled flaxseed offered to non-lactating cattle. Similarly, Sterk et al. (2012) reported higher omasal flows of C18:3 n-3 when animals were fed extruded flaxseed compared to crushed flaxseed, or formaldehyde-treated flaxseed.

Feeding intact oilseeds has also been suggested to provide partial protection of the dietary fatty acids from biohydrogenation. Oba et al. (2009) reported lower levels of biohydrogenation intermediates in the milk of cows fed whole flaxseed compared to rolled flaxseed and attributed these results to the intact oilseed coat being resistant to microbial degradation. Similarly, Mustafa et al. (2003) reported no advantage to feeding micronized flaxseed over whole flaxseed when evaluating animal performance, nutrient utilization and the fatty acid profile of the milk. Although intact oilseeds may be resistant to ruminal biohydrogenation, the digestibility of these products in the lower digestive tract may limit the transfer of dietary fatty acids into milk. Studies have reported lower total tract digestibility when whole flaxseed was fed to dairy cattle (Martin et al., 2008; Petit et al., 2005). Conversely, others have reported similar digestibility when comparing cows fed control treatments (no supplemental oilseed) to those fed whole

oilseeds (Petit, 2003; Petit et al., 2004). The digestibility of the whole oilseeds in the lower gastrointestinal tract may be dependent on the physical properties of the seed (Petit, 2010).

2.4.1.2 Flaxseed Supplementation

Developing feeding strategies that include flaxseed (*Linum usitatissium*) products is of particular interest for increasing the concentration on PUFAs in milk. Compared to other oilseeds commonly grown in North America, flaxseed contains the highest concentration of C18:3 n-3 (Table 2.2). In addition to the desirable fatty acid profile, flaxseed is a source of energy protein and fibre with 40% oil, 20% crude protein, and 30% neutral detergent fibre (**NDF**; Petit et al., 2002; Petit, 2003).

2.4.1.3 Effect of Flaxseed on Milk Production and Dry Matter Intake

Supplementing dairy rations with flaxseed appears to have a neutral effect on milk production regardless of the supplement's physical form. A recent study by Petit et al. (2015) reported similar milk yields when the rations of 32 early-lactating Hostein cows were supplemented with either 50, 100 of 150 g/kg DM of whole flaxseed. Similar results have been reported when feeding whole flaxseed up to 15% of dietary DM in mid- and late-lactating animals (Petit and Gagnon, 2009; Martin et al., 2008). Simiarily, physical processing of flaxseed has been shown to have little to no effect on milk production. Neveu et al. (2013) found no differences in milk production when cows were supplemented with an extruded flaxseed product compared to a control diet with no flaxseed supplementation. Additionally, Sterk et al. (2014) compared the effects of different dietary flaxseed sources (crushed flaxseed, extruded flaxseed and formaldehyde-treated flaxseed) on milk production in early-lactating cattle and reported no differences among treatments.

Table 2.2: Comparison of fatty acid composition of different oilseed crops (% total fatty acids)[1]

	Oilseeds		
	Canola	Flaxseed	Soybeans
C18:0 (Stearic)	1.65	3.20	4.3
C18:1 (Oleic)	61.5	18.2	22.2
C18:2 n-6 (Linoleic)	19.3	14.8	52.6
C18:3 n-3 (α-linolenic)	9.93	57.9	9.23

[1]Adapted from Canadian Grain Commission 2014 harvest report.

Flaxseed supplementation has also been reported to have minimal impact on DMI in lactating cows. Supplementing whole flaxseed at levels up to 15% DM have been shown to have little to no effect on DMI in early- (Petit, 2002) and mid-lactating animals (Secchiari et al., 2003). However, Petit et al. (2015) did report a tendency for DMI to decrease in early-lactating dairy cows with increasing dietary inclusions of whole flaxseed. Processing flaxseed may increase ruminal availability of PUFA which could subsequently impair fibre digestion and feed intake (Petit, 2010). According to Martin et al. (2008) whole flaxseed is less likely to elicit a negative response to DMI compared to processed flaxseed supplements due to the slow release of PUFA in the rumen. However, Gonthier et al. (2005) saw no effect on DMI when comparing intakes of late-lactating cows supplemented with extruded flaxseed at 12% DM compared to control diet with no flaxseed supplementation. More recent studies support the findings of Gonthier et al (2005) reporting no effect on DMI when animals were supplemented with either crushed, extruded, or formaldehyde-treated flaxseed products up to 15% of dietary DM (Neveu et al., 2013; Ferlay et al., 2013; Sterk et al., 2014).

2.4.1.4 Effect of Flaxseed on Milk Composition

The effect of flaxseed supplementation on milk fat composition is highly variable (Table 2.3). In a study conducted by Petit et al. (2009), supplementing dairy rations with 11.1% DM of whole flaxseed had no effect on milk fat percentage or yield. Conversely, milk fat yield was reported to have increased when whole flaxseed was fed at 5, 10 and 15% DM compared to animals with no flaxseed supplementation (Petit and Gagnon, 2009). Similar results were reported by Gonthier et al. (2005) when raw, micronized, or extruded flaxseed products were offered at 10% of dietary DM. On the other hand, Martin et al. (2008) reported a decrease in milk fat concentration from 4.11 to 3.53% when extruded flaxseed was offered to late-lactating

cows at 14.8% dietary DM. The effect of flaxseed supplementation on milk fat appears to be highly dependent on the level of protection of the fatty acids from the biohydrogenating microflora within the rumen. Feeding flaxseed oil form increases the risk of milk fat depression due to extensive biohydrogenation and production of *trans* CLA intermediates associated with MFD (Martin et al., 2008).

Milk protein does not appear to be greatly influenced by supplementation of flaxseed in a cow's diet. Several studies have reported similar milk protein concentrations when flaxseed was included in the diet, regardless of processing (Cortes et al., 2010; Petit et al., 2009; Soita et al., 2003) with few reporting significant changes. Petit et al. (2015) reported a decrease in milk protein concentrations with increased dietary inclusion of whole flaxseed; however, the decrease may have been associated to the progression of the animal's lactation cycle. Neveu et al. (2013) also reported a decrease in milk protein when animals were supplemented with extruded flaxseed at 9% of dietary DM of a high forage diet. On the other hand, Petit et al. (2004) reported a 5.2% increase in milk protein content when whole flaxseed was included in the diet of early lactating dairy cows.

Lactose concentrations in bovine milk have been reported to increase with flaxseed supplementation. Petit et al. (2015) reported increasing levels of milk lactose when the proportion of dietary whole flax was increased in the diet from 50 to 100 g/kg DM. Similar results have been reported throughout the literature (Petit and Cortes, 2010; Gonthier et al., 2005). March et al. (2013) indicated that feeding extruded flaxseed to dairy cattle increased gene expression for glucose transporter 2 which may increase liver secretion of glucose. In addition to increase glucose transporter activity, C18:3 n-3 has been shown to induce high rates of

Table 2.3: The percentage change in milk components when comparing cattle fed flaxseed supplemented diets to non-supplemented diets.

Flaxseed	Inclusion	% Change from control			
		Milk	Milk	Milk	
From	% DM	Fat	Protein	Lactose	Reference
Whole	1.00	-0.60	0.90	NR[1]	Soita et al., 2003
Whole	9.70	4.00	-1.40	NR	Petit et al., 2004
Whole	4.20	-2.70	-0.28	1.05	Côrtes et al., 2010
Ground	12.7	5.20	1.30	1.10	Gonthier et al., 2005
Ground	5.00	-0.22	-1.45	-0.21	Resender et al., 2015
Extruded	4.25	2.62	-1.29	-0.21	Oeffner et al., 2013
Extruded	8.30	1.90	-0.32	0.00	Oeffner et al., 2013
Extruded	11.8	1.67	-0.64	0.41	Oeffner et al., 2013
Extruded	10.0	1.36	-1.27	0.00	Neuveu et al., 2014
Extruded	10.0	2.19	0.32	-0.21	Neuveu et al., 2014
Extruded	12.7	-6.81	-2.35	0.00	Gonthier et al., 2005
Micronized	12.6	3.66	-0.29	0.00	Gonthier et al., 2005
Micronized	1.00	0.60	0.00	NR	Soita et al., 2003
Oil	1.90	-28.79	0.28	1.05	Côrtes et al., 2010
Oil	5.00	0.62	NR	NR	Chillard et al., 2009

[1]NR: Not reported

gluconeogenesis (Mashek and Grummer, 2003). Therefore, higher rates of gluconeogenesis in combination with elevated gene expression for glucose transporter 2 in the liver may increase liver secretion of glucose and make it available for lactogenesis within the mammary gland (Petit et al,. 2015).

2.4.1.5 Effect of Flaxseed on Milk Fatty Acid Composition

Supplementing dairy rations with flaxseed has been shown to decrease the concentration of short and medium chain fatty acids found in milk (C4:0 to C14:0) as well as some C16:0 fatty acids. Glasser et al. (2008) conducted a meta-analysis evaluating the results of 145 oilseed supplementation experiments (including flaxseed) on milk composition. In that study, it was suggested that the decrease in the short and medium chain fatty acids in diets supplemented with UFA can be compensated for by an increase in C18:0 in the milk. In addition, no significant effect on milk fat content was reported in the analysis of the data (Glasser et al., 2008). Decreasing the concentration of short- and medium-chain fatty acids can be considered beneficial to consumers as it may lower the concentration of specific SFA associated with human health concerns.

Decreasing the extent of biohydrogenation could result in the accumulation of beneficial intermediates used for CLA synthesis in the mammary gland. A meta-analysis conducted by Glasser et al. (2008) evaluated the results of 426 experimental oilseed treatments and found a linear increase in the concentrations of *trans* C18:1 and CLA isomers in the milk when whole flaxseed or flaxseed oil was included in the diet compared to the control diets. It has been estimated that 60-98% of the *cis-9, trans-11* CLA recovered in bovine milk originates from the desaturation of trans C18:1 (arising from ruminal biohydrogenation of UFA) in the mammary gland (Griinari and Bauman 1999). Therefore, limiting ruminal biohydrogenation processes to

maximize the synthesis of *trans* 18:1 isomers would be beneficial for increasing the concentration of CLA in the milk.

The concentration of C18:3 n-3 recovered in milk can be increased through the supplementation of flaxseed when compared to conventional feeding systems. However, the extent to which these fatty acids are recovered in the milk is highly dependent on the dietary inclusion of flaxseed and the form in which it is offered. Neveu et al. (2013) reported a 100 % increase in C18:3 n-3 fatty acids in cows fed extruded flaxseed compared to those fed a control diet (with no flaxseed supplementation). However, Chillard et al, (2009) reported no difference in milk C18:3 n-3 concentrations of milk when a whole flaxseed treatment was compared to a control diet with no flaxseed. Total PUFA are also found to increase with flaxseed supplementation; however, it is unusual for these levels to surpass 5% of total fatty acids (Vargas-Bello-Pérez and Garnsworth, 2015).

Earlier sections of this review discussed the ability for mammals to convert C18:3 n-3 into very long chain PUFAs such as C20:5 n-3, and C22:6 n-3. Therefore, increasing the availability of C18:3 n-3 to the host animal would be expected to increase the concentration of C20:5 n-3 and C22:6 n-3 in the plasma and subsequently in the animal's milk. However, increasing the concentrations of C20:5 n-3 and C22:6 n-3 in bovine milk remains a challenge even in instances when dietary sources of these fatty acids are provided instead of flaxseed. While biohydrogenation remains a partial explanation for the lower transfer efficiency, the partitioning of these fatty acids into various physiological uses within the body appears to play a greater role. Studies have found that C20:5 n-3 and C22:6 n-3 are often packaged into cholesterol esters and phospholipids which are not the preferred forms of fat used by the mammary gland (Lock and Bauman, 2004). This preferential packaging of C20:5 n-3 and C22:6 n-3 may limit

incorporation of these fatty acids into the milk since the mammary gland prefers esterified lipids in the form of chylomicrons and very-low-density lipoproteins (Lock and Bauman , 2004). Interestingly, the transfer efficiency of C22:5 n-3 into the milk is much higher than C20:5 n-3 and C22:6 n-3 at 20-30% (Lock and Bauman 2004); however, explanations for these differences remain unclear.

2.4.2 Effect of Condensed Tannins on Milk Fat Composition

Tannins are naturally occurring phenolic compounds found in many plant materials and are commonly divided into two categories: 1) hydrolysable tannins, and 2) condensed tannins (Figure 2.7). Historically, these compounds were considered as "anti-nutritional compounds" due to their affinity to complex with dietary proteins and their potential to limit an animal's dry matter intake (Patra and Saxena, 2011). However, recent research suggests that dietary inclusion of condensed tannin may help protect dietary lipids from complete biohydrogenation and potentially increase the levels of CLA formed in the bovine mammary gland (Kronberg et al., 2007).

Condensed tannins have gained attention for their role in manipulating ruminal lipid metabolism. Several *in vitro* studies have demonstrated the ability of condensed tannins to inhibit complete the biohydrogenation of PUFA. Kronberg et al (2007) reported minimal saturation of C18:3 n-3 when condensed tannins were present. Other studies saw no effect on the disappearance of C18:3 n-3 but, did report an accumulation of *trans*-18:1 fatty acids along with a decrease in C18:0 production. These findings suggest that condensed tannins may have an inhibitory effect on the final step of biohydrogenation (Figure 2.8). Accumulation of trans-18:1 isomer in the rumen could potentially increase its absorption at the small intestine, thus increasing its availability for the synthesis of CLA in the mammary gland (Figure 2.7).

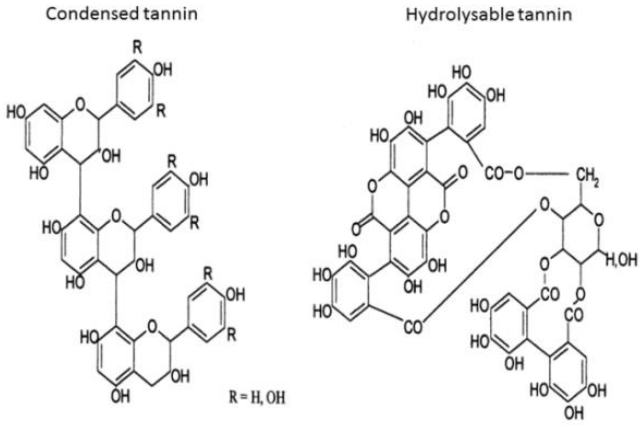


Figure 2.7: Chemical structure of condensed tannins and hydrolysable tannins (McSweeney et al., 2001).

According to Vasta et al. (2009), the ability of condensed tannins to inhibit complete biohydrogenation of PUFAs is not accomplished solely through the inhibition of isomerase activities. Condensed tannins may also impair microbial activity by depriving the microbes of iron, impairing their membrane function, or binding to microbial enzymes and rendering them inactive (Patra and Saxena, 2011).

The effectiveness of condensed tannins in protecting dietary lipids from biohydrogenation is not yet clear since many of the *in vitro* and *in vivo* studies have produced conflicting results. Cenchaar and Chouinar (2009) found no difference in milk fatty acid profiles when dairy cattle were supplemented with 15 g/d of condensed tannins. Buccini et al. (2015) on the other hand, saw an increase in C18:2 n-6 and *trans*-11 C18:1 in the milk of dairy ewes when comparing diets supplemented with soybeans with or without chestnut and quesbrancho tannins. The opposing results reported between *in vitro* and *in vivo* studies may be related to inclusion levels, source of condensed tannins and potential interactions with other dietary components. Application of condensed tannins in feeding management programs has the potential to improve bovine milk fat composition by increase CLA isomers; however, it is clear

that more *in vivo* research must be conducted in order to develop strategies for successful commercial application.

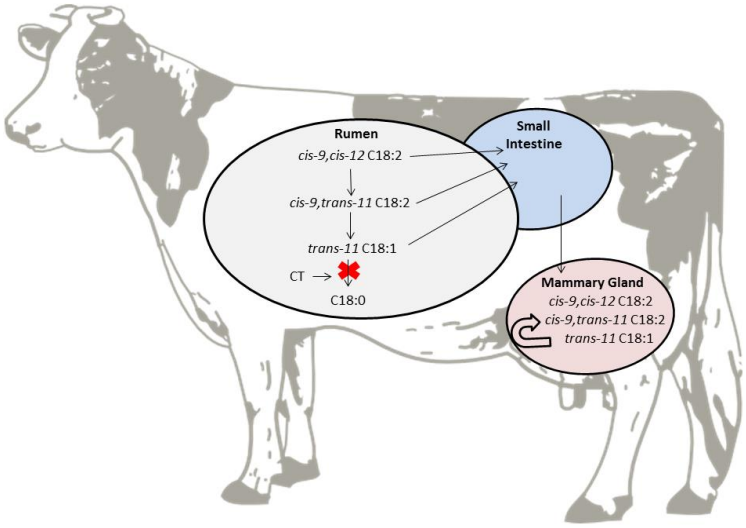


Figure 2.8: Impact of condensed tannins (CT) on biohydrogenation of linoleic acid in the rumen and conjugated linoleic acid synthesis in the mammary gland (Modified from Patra and Saxena, 2011)

2.4.3 Effect of Forage-to-concentrate Ratio on Milk Fat Composition

The impact of forage-to-concentrate ratio on milk fat composition is largely related to the availability of rapidly fermentable carbohydrates in the rumen and the pH of the rumen environment (Sterk et al., 2012). The availability of rapidly fermentable carbohydrates in the rumen may impact the ruminal environment by lowering the pH and causing a shift in the microbial populations (Glasser et al., 2008). Any changes in microbial population have the potential to impact the production of ruminal fermentation end products as well as ruminal lipid metabolism pathways. According to Nielson et al. (2006) high grain diets promote the growth of the ruminal bacterial species *M. elsdenii* and lower the ruminal pH. The lower pH in combination with decreased availability of fibre sources causes a decline in the cellulolytic organism population, particularly in the species *B. fibrisolvens* (Klieve, 2003). Although both *M. elsdenii*

and *B. fibrisolvens* are capable of biohydrogenation of C18:2 n-6 and C18:3 n-3, they use alternative pathways resulting in an increase in the *trans-10, cis-12* CLA which is associated with MFD.

The changes in the ruminal environmental associated with different forage-to-concentrate ratios may also impact the fatty acid profile of the milk. Grain feeding has been associated with a decrease in short- and medium-chain fatty acids and an increase in C18:0 (Jenkins 2003). The concentration of CLA recovered in the milk will also be impacted due to the shift in biohydrogenation pathways limiting the concentrations of *cis-9 trans-11* CLA which has been linked to many human health benefits (Palmquist et al., 2005). Although feeding high grain diets to dairy cattle may decrease the concentration of short- and medium- chain fatty acids in the milk, the risk of MFD and lower synthesis of *cis-9, trans-11* isomers do not make this an ideal feeding strategy.

2.5 Summary

Bovine milk represent a key component of the North American diet with an estimated 12% of human dietary fat originating from dairy products (Zaripheh and Miller, 2008). As such, there is growing awareness of the fatty acid composition of bovine milk and its potential implication on human health. More specifically, there is interest in developing strategies to increase the concentrations of omega-3 PUFA and CLA while simultaneously lowering the concentrations of SFA in bovine milk. Supplementing dairy cow diets with flaxseed has been shown to increase both PUFA and CLA the milk. Furthermore, feeding condensed tannins in a diet supplemented with flaxseed has the potential to increase ruminal production of CLA or CLA substrates by inhibiting complete biohydrogenation of dietary PUFAs. However, feeding dairy cattle diets with elevated levels of PUFA increases the risk of impaired fibre digestibility in the

rumen, altered ruminal fermentation, MFD and poor animal performance which may subsequently have economic implications to the producer. Both intact flaxseed and extruded flaxseed have been shown to provide partial protection of dietary PUFA from the rumen environment and, thereby, mitigating the risk factors mentioned above; however, it remains unclear as to which form may be superior when evaluating ruminal metabolism, milk fat composition, and animal performance. Furthermore, to our knowledge no study has compared the effects of feeding extruded flaxseed supplements with varying levels of condensed tannins on the same variables of metabolism and animal performance. Evaluating the effects of whole versus extrusion as well as the effects of extrusion and condensed tannins may provide valuable insight for the development of flaxseed feeding programs in dairy rations.

2.6 Hypothesis

Supplementing dairy rations with extruded flaxseed compared to whole flaxseed can improve the concentrations of healthy lipids in bovine milk by increasing omasal flow of PUFA without negatively impacting rumen fermentation, omasal flow of nutrients, animal performance, or milk composition. Furthermore, elevating the concentration of condensed tannins in an extruded flaxseed product will provide an additive effect on increasing healthful fatty aicds in the milk.

2.8 Objectives

The objective of this research was to evaluate the effects extruded flaxseed on omasal flow of nutrients, rumen fermentation, animal performance and milk fat composition in lactating dairy cattle compared to whole flaxseed. Furthermore, the effects of extruded flaxseed with elevated levels of condensed tannins will also be evaluated using the same parameters.

3.0 MATERIALS AND METHODS

All experimental procedures used in this study were approved by the University of Saskatchewan Animal Care Committee (UCACS Protocol No. 20040048) and were conducted in accordance with the Canadian Council of Animal Care guidelines (1993).

3.1 Experimental design, animal description and housing

Eight multiparous, mid-lactating Holstein cows (712.7 ± 92.3 kg BW; 116.5 ± 17.5 DIM at the beginning of the experiment) from the University of Saskatchewan's Greenbrae herd were used in a replicated 4 x 4 Latin square experimental design. Each period consisted of 28 days with 20 days of dietary adaptation and 8 days of sample and data collection. The dairy cows were housed indoors at the Rayner Dairy Research and Teaching Facility (University of Saskatchewan, Saskatoon, SK) in individual tie-stalls. Four of the cows in one Latin square were fitted with permanent ruminal cannulas to facilitate ruminal fluid and omasal digesta sample collections. Cows were milked three times daily at 0430, 1230 and 1900 h and body weights were recorded on d 1 through 3 of each period.

3.2 Flaxseed ingredients, experimental diets and feeding management

All flaxseed supplements were manufactured by O&T Farms Ltd. (Regina, SK, Canada) and their ingredient compositions are presented in Table 3.1. Flaxseed supplements were formulated to have similar ingredient compositions and to only vary in either processing methods or levels of condensed tannins. The whole flaxseed product (designated **RAW**) had the same ingredient profile as the commercially available extruded flaxseed product linPRO-R ® (O&T Farms Ltd., Regina, SK, Canada; designated **LPR**). To increase the concentration of condensed tannins in an extrude flaxseed product, ground field peas in the LPR product were substituted for groun, high-tannin faba beans (designated **LPF**) The extruded products LPR and LPF were manufactured using single-screw dry extrusion technology

Table 3.1: Ingredient composition of flaxseed supplements fed to lactating Holstein cows

	Flaxseed supplement[1]		
Ingredient, % DM	RAW	LPR	LPF
Whole flaxseed	54.7	54.7	54.7
Ground field peas	37.8	37.8	-
Ground faba beans[2]	-	-	37.8
Dehydrated alfalfa	6.97	6.97	6.97
Vitamin E[3]	0.10	0.10	0.10
Mold inhibitor[4]	0.30	0.30	0.30
Ethoxyquin[5]	0.05	0.05	0.05

[1]Supplement: RAW = non-extruded flaxseed supplement; LPR = extruded flaxseed and pea supplement (LinPRO-R®); LPF = extruded flaxseed and faba bean supplement. All flaxseed supplements were manufactured by O&T Farms Ltd (Regina, SK.).

[2]Faba bean variety: Malik 9-4

[3]Vitamin E: Microvit® (min 500 IU/g; Adisseo,; Alpharella, CA)

[4]Mold inhibitor: No Mold85 (85% propionic acid; Agri-Marketing Corp.; Mont. St. Hilaire, QB)

[5]Ethoxyquin: Santoquin® (min. 91% ethoxyquin; Novus International, Inc.; St. Charles, MO.)

(InstaPRO® Model 2500) with barrel temperatures averaging 120°C. Flaxseed supplements were sampled before and after extrusion processing and samples were stored at room temperature for later determination of condensed tannin concentrations.

Experimental diet composition is presented in Table 3.2. Experimental diets were offered to the cows as a TMR with a 50:50 forage-to-concentrate ratio (DM basis). Experimental treatments consisted of either a control diet (**CTL**) with no addition of a flaxseed supplement or three diets that contained one of three flaxseed supplements. For diets containing flaxseed supplements, a 1:1 substitution of 3 kg of the CTL concentrate was made for either a non-extruded flaxseed and pea product (designated **RAW**), an extruded flaxseed and pea product (LPR), or an extruded flaxseed and high-tannin faba bean product (LPF). The three treatment diets were formulated to achieve an ether extract **(EE)** level approaching, but not exceeding, 6% of TMR dry matter (**DM**) in accordance with the NRC Dairy (2001) recommendations. Cows were fed twice daily at 0930 and 1700h with *ad libitum* access to feed and water.

3.3 Data Collection and Sampling

The triple indigestible marker technique (France et al., 1986) was used to determine omasal nutrient flow from the four ruminally-cannulated cows in one of the two Latin squares. Chromium (**Cr;** Udén et al., 1980) and ytterbium (**Yb**; Siddons et al., 1985) were used as fluid particulate (**FP**) phase and small particulate (**SP**) phase digesta markers, respectively. Indigestible neutral detergent fibre (**iNDF**; Reynal et al., 2005) was used as a large particulate (**LP)** phase marker. Furthermore, a solution of $(^{15}NH_4)_2SO_4$ with 10 atom percentage excess (**APE**) was prepared according to Reynal et al. (2005) and infused simultaneously with the Yb solution for later determination of microbial protein production, using ^{15}N as a marker.

Table 3.2: Ingredient composition of experimental diets fed to lactating Holstein cows

	Diet[1]			
Ingredient composition, % DM	CTL	RAW	LPR	LPF
Barley silage	28.2	28.1	28.1	28.1
Alfalfa hay	20.0	20.0	20.0	20.0
Ground corn grain	9.10	7.12	7.12	7.12
Pea grain	3.83	2.99	2.99	2.99
Ground barley grain	23.8	18.55	18.55	18.55
Canola meal Solvent	3.86	3.02	3.02	3.02
Soybean meal solvent	4.26	3.33	3.33	3.33
Corn gluten meal	0.985	0.770	0.770	0.770
Corn distillers	1.67	1.30	1.30	1.30
Mineral-Vitamin Premix[2]	1.23	0.963	0.963	0.963
Palmitic acid	0.718	0.561	0.561	0.561
Molasses cane	0.749	0.586	0.586	0.586
Biotin[3]	0.037	0.029	0.029	0.029
R-choline[4]	0.220	0.172	0.172	0.172
K-Mg-S	0.084	0.066	0.066	0.066
Sodium bicarbonate	0.525	0.411	0.411	0.411
Limestone	0.560	0.438	0.438	0.438
Niacin	0.021	0.017	0.017	0.017
Ethoxyquin[5]	0.001	0.009	0.009	0.009
Salt	0.228	0.178	0.178	0.178
RAW	-	11.4	-	-
LPR	-	-	11.4	-
LPF	-	-	-	11.4

[1] Diets: CTL = Control diet with no flaxseed supplement; RAW = diet supplemented with a non-extruded flaxseed and pea supplement; LPR = diet supplemented with an extruded flaxseed and pea supplement (linPRO-R ®); LPF = diet supplemented with an extruded flaxseed and high tannin faba bean supplement. All flaxseed supplements were manufactured by O&T Farms Ltd (Regina, SK.).

[2]Mineral-Vitamin Premix contained: 16% DM Ca, 7% DM P, 7% DM Mg, 2% DM K, 10% DM Cl, 1.25% DM S, 1507 ppm Mn, 678 ppm Cu, 1005 ppm Fe, 2513 ppm Zn, 80 ppm I, 30 ppm Co, 20 ppm Se, 251 256 IU/kg Vit A, 80 402 IU/kg Vit. D3, 2010 IU/kg Vit E.

[3]Biotin: 2% biotin source

[4]R-Choline: 25% choline source

[5]Ethoxyquin: Santoquin ® (Novus International, Inc.; St. Charles Missouri)

Prior to the initiation of marker infusions (d 13), a 500 mL sample of ruminal digesta was collected for later determination of background ^{15}N enrichment (**NB**). A priming dose of the two marker solutions-equivalent to half the daily dose-was administered into the rumen following NB collection. Marker solutions were continuously infused from d 13 to 23 at a rate of 1 L per day using a peristaltic pump (Model 205U, Watson and Marlow, Cornwall, UK) infusing 2.77 g of Cr (Binnerts et al., 1968) 3.35 g of Yb (Brito et al., 2007) and 0.22 g of ^{15}N (Brito et al,. 2006) into the rumen of each cow.

The omasal sampling technique was used for the collection of omasal digesta (Huhtanen et al., 1997). A sampling tube was inserted into the omasal orifice by hand via the ruminal cannula. Once inserted, a 450 mL sample of omasal digesta was aspirated from the omasal canal using a custom-made pulsatile vacuum pump. Omasal digesta samples were collected at 0600, 1200 and 1800 h on day 20, 0000, 0800, 1400 and 2000 h on day 21, 0200, 1000, 1600 and 2200 h on day 22 and 0400 on day 23. Each sample was mixed thoroughly and partitioned into 100-mL, 125-mL, and 200-mL sub-samples. The 100-mL and 200-mL sub-samples were pooled by cow and treatment to form 2.4-L and 1.2-L composite samples, respectively, which were stored at -20^{o}C pending indigestible marker analysis. The remaining 125 mL of omasal digesta was held on ice and pooled by cow over two collection times to yield a 250-mL aliquot. The sampling tube was removed and re-inserted at each sampling time to mitigate potential problems of restricted digesta passage from the rumen and incorrect positioning of the tube itself.

The 250 mL aliquots of omasal digesta were designated for isolation of particle associated bacteria (**PAB**) and fluid associated bacteria (**FAB**). Samples were first squeezed through two layers of cheesecloth and washed with 250 mL of 0.85% NaCl. The filtrate was collected in a 1,000-mL container labeled for FAB isolation. The solids were immediately

transferred to a 1000-mL container labeled for PAB isolation containing 175 mL of a chilled 0.85% NaCl (wt/vol) and 0.1% Tween-80 (vol/vol) solution. Containers containing FAB and PAB samples were held on ice and immediately transferred to the laboratory for FAB and PAB isolation according to Brito et al. (2009). For each collection period, isolated FAB and PAB pellets were composited based on cow and treatment and stored in aluminum trays at -20°C pending further analysis.

To quantify dietary effects on ruminal fermentation characteristics, 1,000 mL of ruminal fluid was collected from each of the four cannulated cows on d 27 and 28. Samples of 250-mL were collected from the cranial-dorsal, ventral-dorsal, caudal-dorsal and ventral-dorsal sac of the rumen at 0900, 1000, 1100, 1200, 1300, 1600, 1900, 2200 h on d 27 and 0100, 0400, and 0700 h on day 28. The four 250-mL samples were combined to form a 1,000-mL aliquot at each sampling time. The 1,000-mL samples were squeezed through four layers of cheesecloth and pH of the filtrate was measured immediately using a portable pH meter (VWR Symphony SP70C). A 10 mL sub-sample of ruminal fluid was preserved in 2 mL of 25% metaphosphoric acid (H_2PO_4) for later determination of short chain fatty acid (**SCFA**) concentrations. Another 10 mL sub-sample of ruminal fluid was preserved in 2 mL of 1% sulfuric acid (H_2SO_4) for later determination of ammonia nitrogen (**NH_3-N**) concentrations. All ruminal fluid samples were stored at -20°C pending analysis.

Milk weights of all eight cows were recorded daily over the 8 day collection period. Milk samples were collected over three consecutive days (26, 27 and 28) and pooled proportionally based on milk weights to form a 1,000-mL aliquot over the three days. The aliquot was then sub-sampled, in duplicate, into plastic vials containing 2-bromo-2-nitropropane-1-2-diol as a preservative before being sent to CanWest DHI (Edmonton, AB, Canada) for analysis of milk

composition. Additional duplicate samples were collected in 40 dram vials and submitted to Lipid Analytical Services Ltd. (Guelph, ON, Canada.) for later determination of fatty acid concentrations.

To determine the chemical composition of the experimental diets fed to the dairy cows, samples of the individual feed ingredients were collected on d 21 through 23. Forage and barley ingredients were dried at 55°C in a forced-air oven and stored at room temperature pending chemical analysis. Orts from all eight cows were collected and weighed daily at 0900 h over the 8 day collection period for determination of individual cow dry matter intake (**DMI**). Samples of orts from the four cannulated cows were collected on d 22 through 24, and pooled by cow and treatment. Due to the high oil content, orts samples were freeze-dried and stored at room temperature pending chemical analysis.

3.4 Sample Analyses

The 2.4 L samples of omasal digesta were thawed at room temperature and separated into LP, SP and FP phases as described by Brito et al. (2009). Thawed samples were squeezed through a single layer of cheesecloth and the solids retained on the cheesecloth were defined as the LP phase. The filtrate was centrifuged at 1, 000 x g for 5 min at 5°C. The supernatant was decanted from the pellet and defined as the FP phase while the pellet was defined as the SP phase. Following separation of the 3 phases, the LP, SP and FP samples were stored a -20°C before being freeze-dried. After freeze drying, the LP phase samples were ground through a 1-mm screen (Christy-Norris mill), and the SP and FP samples were ground with a mortar and pestle.

Concentrations of Cr, Yb and iNDF markers were quantified to facilitate reconstitution of omasal true digesta (**OTD**). The concentrations of Cr and Yb were determined according to

Vincent et al. (2004). Samples (1-g) of LP, SP and FP phases were ashed overnight at 550°C followed by nitric acid digestion using 1.57 M nitric acid containing 2 g of KCl/L. Digested samples were then diluted to 100-mL with distilled water before Cr and Yb analysis using Thermo Scientific iCE 3300 atomic absorption spectrophotometer (Thermo Fisher Scientific, 81 Wyman Street, Waltham, MA, 02454). The concentration of iNDF was determined according to Ahvenjärvi et al. (2000) by weighing LP, SP , feed and orts samples in triplicate into 5 × 10 cm nylon mesh bags (6 µm pore size; part no. 03-6/5, Sefar America Inc., Depew, NY) at 1.5 g, 2.0 g and 3.0 g, respectively. Bags were assigned at random for incubation in the rumen of 4 cannulated cows for 12 d. Immediately following incubation, the bags were rinsed in cold water for 30 min and dried for 48 h at 55°C in a forced air oven. The dried bags were weighed and analyzed for iNDF content according to Ankom method 6 (2011) with alpha amylase (17,400 LU/ml) and sodium sulfite (0.5 g/50 mL of neutral detergent solution). The mean concentration of Cr was 7 and 61 times greater in the FP compared to the SP and LP, respectively. The mean concentration of iNDF was 5 times greater in the LP compared the SP. The mean concentration of Yb was 1.3 and 3.4 times greater in the FP compared the SP and LP, respectively. These results suggested Yb marker dysfunction as it did not associate with the proper digesta phase. As a result, the concentrations of the three markers could not be used to physically reconstitute the OTD using the triple marker technique. Therefore, whole omasal digesta (**WOD**) was reconstituted based on the dry matters of the FP, SP and LP and the single marker technique was applied using iNDF as the sole indigestible marker (France and Siddons, 1986).

Compositional analysis of reconstituted WOD was conducted to determine nutrient flow from the rumen. Samples were analyzed for DM (AOAC, 2006; method 930.15), ash (AOAC, 2000; method 942.05), NDF (Ankom method 6, 2011) and acid detergent fibre (**ADF**; AOAC,

2000; method 973.18). To determine concentrations of NH_3-N samples of 0.5g WOD were brought into solution by adding 10 mL of 0.07 *M* sodium citrate and vortexing the sample mixture (Broderick and Kang, 1980). The sample mixture was then incubated in a forced air oven at 39°C for 30 minutes before centrifuging the extracts at 18,000 x g for 15 min at 4°C (Broderick and Kang, 1980). The resulting supernatant was then used for analysis of NH_3-N concentrations in WOD using the phenol-hypochlorite method (Broderick and Kang, 1980).

Samples of NB, FAB, PAB and WOD were prepared for ^{15}N analysis as described by Brito et al. (2009). Samples of NB, FAB, PAB and WOD (2-mg) were weighed into 8 x 6-mm tin capsules (Elemental Microanalysis Limited, Okehampton, UK) and 50 µL of 72 mM K_2CO_3 was added to each capsule. Capsules were then incubated in a forced air oven at 60°C for 24 h to volatilize any NH_3-N in the sample. Following NH_3-N volatilization, enrichment of ^{15}N in non-ammonia nitrogen (**NAN**) was determined through the combustion of N_2 gas in a Costech ECS4010 elemental analyzer (Costech Analytical, California, USA) coupled with a Delta V Advantage mass spectrometer with a Conflo IV interface (Thermo Scientific, Bremen, Germany).

Ruminal fluid samples preserved in 1% H_2SO_4 were analyzed for NH_3-N using the phenol-hypochlorite method as described by Broderick and Kang (1980). Ruminal fluid samples preserved in 25% H_2PO_4 were analyzed according to Khorasani et al. (1996) for SCFA by gas chromatography using and Agilent 6890 system (Mississauga, ON, Canada) fitted with a Zebron ZB-FFAP capillary column (0.32mm x 0.25µm, Phenomenex, Torrance, USA) Zebron and an Alegient 7863 series injector (Mississauga, ON, Canada).

Milk component analysis was conducted by CanWest DHI laboratories (Edmonton, AB, Canada). The concentration of milk fat, protein, lactose and milk urea nitrogen (**MUN**) were

analyzed using an infrared analyzer (Foss System 4000, Foss Electric, Hillerød, Denmark) according to AOAC (1990) method 972.16.

Dried feed ingredients and orts were ground through a 1-mm screen (Christy-Norris mill) and submitted to Cumberland Valley Analytical Services (Haggerstown, MD, USA) for compositional analysis. Samples were analyzed for DM (AOAC, 2006; method 930.15), crude protein (**CP**; AOAC, 2000; method 990.03), ADF (AOAC, 2000; method 973.18), ash (AOAC, 2000; method 942.05), starch (Hall, 2009) and NDF (Van Soest, 1991). The Van Soest (1991) NDF analysis was modified to use Whatman 934.AH glass micro-fibre filters with 1.5 μm particle retention. Energy values were calculated as specified by Cumberland Valley Analytical Services using the Ohio State Summative Equations (Weiss, 1988). Samples of flaxseed ingredients were submitted to Lethbridge Research Centre (Lethbridge, AB) for determination of condensed tannins using the acid-butanol assay (Porter et al., 1986). The chemical analyses of individual feed ingredients were then used to calculate the chemical composition of experimental diets.

Crude fat and fatty acid analysis of feed ingredients, orts, milk samples and omasal digesta was conducted in duplicate by Lipid Analytical Services Ltd. (Guelph, ON, Canada). Fat extraction was conducted according to Bligh and Dyer (1959) and fatty acid methyl-esters were prepared according to AOCS (2009) method CE-66. Fatty acid analysis was carried out using a Varian 3400 cx gas chromatography unit with a Varian 8200 injector (Allegiant Technologies; Mississauga, ON, Canada) according to AOAC (2001) method 996.06 using tritridecanoin (C13:0) as an internal standard.

3.5 Calculations and Statistical Analysis

The flow of nutrients (**F_N**) at the omasal canal was calculated according to the single marker method (France and Siddons, 1986) based on the assumption that omasal samples obtained were representative. Where $C_{N,X}$ (mg/g digesta) is the concentration of a nutrient constituent in WOD, I_M (mg/d) is the infusion rate of indigestible marker and $C_{M,X}$ (mg/d) is the concentration of the indigestible marker within the sample.

$$F_N = C_{N,x}I_M / \ CM.x$$

The flow of nitrogen constituents at the omasal canal were calculated according to Brito et al. (2009). Enrichment of ^{15}N calculated as the difference between sample and NB ^{15}N atom % values.

$$^{15}N \text{ APE} = {}^{15}N \text{ atom \%} - \text{NB } {}^{15}N \text{ atom \%}$$

The flows of FAB and PAB NAN could not be calculated because the individual omasal digesta phases could not be separated and the flow of FAB and PAB are assumed to be represented by the flows of the FP and PF , respectively. Therefore total bacterial NAN was calculated using an average of FAB and PAB ^{15}N APE values.

$$\text{Total bacterial NAN flow} = (\text{Mean WOD NAN flow}) * \frac{(\text{Mean WOD } {}^{15}N \text{ APE})}{(\text{Mean FAB \& PAB } {}^{15}N \text{ APE})}$$

The omasal flow of non-ammonia-non-microbial-nitrogen (**NANMN**) was calculated as:

$$\text{NANMN flow} = \text{total NAN flow} - \text{total bacterial NAN flow}$$

The total flow of NAN was assumed to consist of bacterial NAN and NANMN. Flows and intakes were expressed in grams per day (g/d) or kilograms per day (kg/d).

Energy corrected milk (**ECM**) yield was calculated according to Orth (1992) using the following formula:

$$\text{ECM} = [0.327 \times \text{milk yield (kg)}] + [12.95 \times \text{fat yield (kg)}] + [7.2 \times \text{protein yield (kg)}]$$

Statistical analysis of production and milk fatty acid data from all eight cows were carried out as a replicated 4 x 4 Latin square design using PROC MIXED (SAS 9.4) according to the model $Y_{ijkl} = \mu + s_i + p_j + \delta_{k(i)} + \alpha_l + \epsilon_{ijkl}$. Where Y_{ijkl} is the dependent variable, μ is the overall mean, s_i is the fixed effect of square i, p_j is the fixed effect of period j, $\delta_{k(i)}$ is the random effect of cow k within square i, α_l is the fixed effect of dietary treatment l, and ϵ_{ijkl} is the residual error. Various variance and covariance structures were tested and the best variance and covariance structure was used for the final analysis of variables. The DDFM = kenwardroger option was used for the approximation of degrees of freedom from the means as described by Kenward and Roger (1997). Contrast statements were used to compare RAW to LPR, LPR to LPF and CTL to the mean of RAW+LPR+LPF. Significance was declared at $P \leq 0.05$ with trends discussed at $0.05 < P \leq 0.10$.

Rumen fermentation characteristics and omasal nutrient flow were analyzed as a 4 x 4 Latin square using PROC MIXED (SAS 9.4) according to the model $Y_{jkl} = \mu + p_j + \delta_k + \alpha_l + \epsilon_{ijkl}$. Where Y_{ijk} represents the dependent variable, μ is the overall mean, , p_j is the fixed effect of period j, δ_k is the random effect of cow k , α_l is the fixed effect of dietary treatment l, and ϵ_{ikl} is the residual error. Various variance and covariance structures were tested and the best variance and covariance structure was used for the final analysis of variables. Repeated measures were accounted for by including periods in the repeated statement of SAS. The DDFM= kenwardroger option was used for the approximation of degrees of freedom from the means (Kenward and Roger, 1997). Contrast statements were used to compare RAW to LPR, LPR to LPF and CTL to the mean of RAW+LPR+LPF. Significance was declared at $P \leq 0.05$ with trends discussed at $0.05 < P \leq 0.10$.

4.0 RESULTS

4.1 Flaxseed supplement characteristics

The chemical compositions of the experimental flaxseed supplements (RAW, LPR and LPF) are presented in Table 4.1 (with an expanded version available in Appendix A). Crude protein, NDF, ADF and ash were similar among flaxseed supplements. The ether extract (**EE**) content was higher in the RAW compared LPR and LPF. Energy values were estimated using the Ohio State Summative Equations (Weiss, 1998) and follow a similar pattern as EE content with RAW having higher energy values compared to LPR and LPF. Condensed tannins were not detected in the RAW or LPR supplements, as was anticipated based on the ingredient composition of the flaxseed supplements. Replacing the peas with high tannin faba beans in the LPF supplements resulted in condensed tannin levels of 1.17 mg/g. It is important to note that the level of condensed tannins was decreased by 83% after extrusion processing (Appendix B).

The fatty acid profile of the flaxseed supplements are presented in Table 4.2. Little variation in the fatty acid profile of the flaxseed supplements was observed. This was expected as the flaxseed supplements contained similar inclusion levels of flaxseed. Total PUFA were similar in RAW, LPR and LPF with C18:3 n-3 representing the majority of the PUFAs in the supplements. However, the C18:3 n-3 content of the RAW supplement reported lower levels compared to LPR and the LPF despite having the same inclusions of flaxseed as an ingredient.

4.2 Diet characteristics

The chemical compositions of experimental diets are presented in Table 4.3 (with an expanded version available in Appendix C). Diets were isonitrogenous with similar NDF, ADF and ash content. The RAW, LPR and LPF diets averaged and EE content of 5.52% while the CTL averaged 3.21% EE. The energy values were higher for the flaxseed treatment diets

Table 4.1: Chemical composition of flaxseed supplements fed to lactating Holstein cows

	Supplement[1]		
Chemical composition	RAW	LPR	LPF
Dry matter, %	90.7	91.2	91.6
Ash, % DM	3.89	4.01	3.92
Crude protein, % DM	22.9	23.6	24.5
Soluble protein, % CP	58.8	46.0	46.0
Neutral detergent fibre, % DM	18.0	19.1	20.9
Acid detergent fibre, % DM	9.23	8.83	9.8
Non fibre carbohydrates, % DM	28.3	34.8	31.0
Starch, % DM	16.3	18.2	18.6
Ether extract, % DM	27.5	22.4	22.5
NE_m[2], Mcal/ kg of DM	1.36	1.26	1.26
NE_g,[3] Mcal/kg of DM	0.985	0.907	0.90
NE_L[4], Mcal/kg of DM	2.45	2.31	2.29
Condensed tannin, mg/g	N/D[5]	N/D[5]	1.17

[1]Supplement: RAW = non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant ; linPRO-R ®); and LPF = extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant. All flaxseed supplements were manufactured by O&T Farms Ltd. (Regina, SK)

[2] NE_m: Net energy of maintenance. Calculated using the Ohio State Summative Equations (Weiss, 1998)

[3] NE_g: Net energy of gain. Calculated using the Ohio State Summative Equations (Weiss, 1998)

[4] NE_L: Net energy of lactation. Calculated using the Ohio State Summative Equations (Weiss, 1998)

[5]ND: Not detected

Table 4.2: Fatty acid composition (% FAME) of flaxseed supplements fed to lactating Holstein cows

	Supplement[1]		
Fatty Acids	RAW	LPR	LPF
C14:0	0.093	0.049	0.046
C14:1	0.001	0.001	0.001
C15:0	0.034	0.022	0.031
C16:0	7.01	6.13	5.84
C16:1	0.092	0.075	0.066
C18:0	3.10	3.30	3.29
C18:1	19.7	21.6	20.7
C18:2 n-6	26.3	21.6	18.8
C18:3 n-3	42.9	46.3	50.4
C20:0	0.217	0.176	0.170
C20:1	0.303	0.269	0.244
C20:2n-6	0.01	0.029	0.244
C20:3n3	0.015	0.029	0.028
C22:0	0.205	0.167	0.027
C24:0	0.084	0.101	0.001
Total SFA[2]	10.7	9.95	9.64
Total MUFA[3]	20.1	22.0	21.1
Total PUFA[4]	69.2	68.1	69.3
Total n-3 PUFA[5]	42.9	46.4	50.5
Total n-6 PUFA[6]	26.3	21.7	18.8
n-6:n-3[7]	0.613	0.468	0.374

[1]Supplement: RAW = non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant ; linPRO-R ®); and LPF = extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant. All flaxseed supplements were manufactured by O&T Farms Ltd. (Regina, SK)

[2]Total SFA = Total BCFA + C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C22:0 + C24:0 + C26:0

[3]Total MUFA = C14:1 + C16:1 + C18:1 + C18:1 + C18:1 + C18:1 + C18:1 + C18:1 + C18:1 + C18:1 + C18:1 + C18:1 + C18:1 + C18:1 + C18:1 + C18:1 + C20:1.

[4]Total PUFA = Total n3 PUFA + Total n6 PUFA

[5]Total n3 PUFA = C18:3n3 + C20:3n-3 + C20:5n3 + C22:3n-3 + C22:5n3.

[6]Total n6 PUFA = C18:2n6 + C18:3n-6 + C20:2n-6 + C20:3n6 + C20:4n6 + C22:4-6.

[7] n6:n3 = ratio of Total n6 PUFA to Total n3 PUFA

Table 4.3: Chemical composition of experimental diets fed to lactating Holstein cows

	Diet[1]			
Chemical Composition	CTL	RAW	LPR	LPF
Dry matter, %	51.4	51.5	51.2	51.6
Ash, % of DM	9.60	9.00	9.02	9.01
Crude protein, % DM	16.3	16.8	16.8	16.9
Soluble protein, % CP	31.6	36.9	35.4	35.4
Neutral detergent fibre, % DM	30.4	30.6	30.8	31.0
Neutral detergent fibre digestibility, % NDF	53.5	51.8	53.1	52.4
Acid detergent fibre, % of DM	19.5	19.6	19.5	19.5
Starch, % DM	28.7	25.7	25.9	26.0
Non-fibre Carbohydrates, % DM	41.6	38.8	39.6	39.1
Ether extract, % DM	3.21	5.91	5.32	5.34
NE_m[2], Mcal/ kg of DM	0.719	0.779	0.732	0.767
NE_g,[3] Mcal/kg of DM	0.445	0.495	0.461	0.486
NE_L[4], Mcal/kg of DM	1.35	1.45	1.44	1.44

[1]Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = diet including a extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant ; linPRO-R ®); and LPF = diet including a extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant). All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK)

[2]NE_m = Net energy of maintenance. Calculated using the Ohio State Summative Equations (Weiss, 1998)

[3]NE_g = Net energy of gain. Calculated using the Ohio State Summative Equations (Weiss, 1998)

[4]NE_L: Net energy of lactation. Calculated using the Ohio State Summative Equations (Weiss, 1998)

Table 4.4: Fatty acid composition (% FAME) of experimental diets fed to lactating Holstein cows

	Diet[1]			
Fatty acid	CTL	RAW	LPR	LPF
C14:0	0.957	0.846	0.840	0.841
C14:1	0.032	0.032	0.032	0.032
C15:0	0.188	0.181	0.180	0.179
C16:0	32.8	29.0	28.9	28.9
C16:1	0.709	0.695	0.692	0.693
C18:0	5.18	4.80	4.82	4.82
C18:1	13.2	13.6	13.7	13.8
C18:2 n-6	29.7	29.3	28.4	28.7
C18:3 n-6	0.005	0.005	0.005	0.005
C18:3 n-3	14.3	18.8	19.7	19.2
C18:4 n-3	0.000	0.001	0.000	0.000
C20:0	0.478	0.477	0.472	0.472
C20:1	0.483	0.446	0.439	0.442
C20:2 n-6	0.002	0.003	0.005	0.006
C20:3 n-6	0.002	0.002	0.002	0.002
C20:4 n-6	0.000	0.000	0.000	0.000
C20:3 n-3	0.000	0.002	0.003	0.003
C20:4 n-3	0.012	0.010	0.010	0.010
C20:5 n-3	0.014	0.014	0.014	0.014
C22:0	0.697	0.703	0.696	0.698
C22:1	0.486	0.402	0.404	0.408
C22:2 n-6	0.000	0.000	0.000	0.000

Table 4.4 (cont'd): Fatty acid composition (% FAME) of experimental diets fed to lactating Holstein cows

	Diet[1]			
Fatty acid	CTL	RAW	LPR	LPF
C22:4 n-6	0.033	0.033	0.034	0.034
C22:5 n-6	0.000	0.000	0.000	0.000
C22:5 n-3	0.016	0.016	0.016	0.016
C22:6 n-3	0.012	0.012	0.012	0.012
C24:0	0.494	0.497	0.500	0.499
C24:1	0.134	0.134	0.134	0.134
Total SFA[2]	40.8	36.5	36.4	36.4
Total MUFA[3]	15.1	15.3	15.4	15.5
Total PUFA[4]	44.1	46.2	48.2	48.1
Total n-3 PUFA[5]	14.4	18.9	19.8	19.3
Total n-6 PUFA[6]	29.7	29.3	28.5	28.8
n-6:n-3[7]	2.07	1.55	1.44	1.49
Total fatty acids (% Ether Extract)	82.5	74.7	88.2	86.8

[1]Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = diet including a extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant ; linPRO-R ®); and LPF = diet including a extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant). All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK)

[2]Total SFA = Total BCFA + C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C22:0 + C24:0 + C26:0

[3]Total MUFA = C14:1 + C16:1 + C18:1 + C20:1

[4]Total PUFA = Total n-3 PUFA + Total n-6 PUFA

[5]Total n3 PUFA = C18:3 n-3 + C20:3 n-3 + C20:5 n-3 + C22:3 n-3 + C22:5 n-3.

[6]Total n6 PUFA = C18:2 n-6 + C18:3 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:4 n-6.

[7]n-6:n-3 = ratio of Total n-6 PUFA to Total n-3 PUFA

compared to the CTL, which is reflective of the high EE content in those diets compared to the CTL diet. Dietary starch content was higher in the CTL diet (28.7% DM) compared to the mean for the RAW, LPR and LPF diets (25.9% DM).

The fatty acid compositions of experimental diets are presented in Table 4.4. The total PUFAs in the RAW, LPR and LPF diets averaged 47.5% (as % of FAME) which was higher than the CTL diet at 44.1%. The CTL diet had lower levels of C18:3 n-3 and C18:2 n-6 compared to RAW, LPR and LPF diets. Total SFA in the RAW, LPR and LPF diets averaged 36.4% which was lower compared to the CTL diet at 40.8%.

4.3 Ruminal Fermentation Characteristics

No sampling time x treatment interactions were observed for ruminal, ammonia nitrogen (**NH_3-N**) concentration or SCFA concentrations; therefore, only mean values for rumen fermentation characteristics are presented (Table 4.5). Ruminal fluid pH and total SCFA concentrations were unaffected by dietary treatment. Acetate concentrations were lower in ruminal fluid of cows fed LPF diet compared to those fed LPR diet ($P = 0.03$). Ruminal fluid concentrations of propionate and butyrate were unaffected by dietary treatment. Concentrations of ruminal fluid NH_3-N were lower in cows fed the CTL diet compared to the average of the Raw-, LPR- and LPF-fed cows ($P = 0.05$).

4.4 Nutrient Intakes, Rumen Digestibility and Omasal Flow

Nutrient intakes and omasal flow of nutrients from the four ruminally-cannulated cows are presented in Table 4.6. Results show that intakes of DM did not differ among treatments and averaged 24.9 kg/d. The intake of OM decreased when the combined average of the RAW, LPR and LPF diets was compared to the CTL diet ($P = 0.05$). Intakes of NDF and ADF were

Table 4.5: Ruminal fermentation characteristics of lactating Holstein cows fed different flaxseed supplements [1]

	Diet[2]					Contrasts: *P*-values		
Item	CTL	RAW	LPR	LPF	SEM	RAW vs. LPR	LPR vs. LPF	CTL vs. RAW+LPR+LPF
Rumen pH	6.07	6.03	6.00	6.03	0.80	0.70	0.70	0.70
Rumen SCFA, m*M*								
Acetate	50.3	50.6	51.2	48.0	0.95	0.69	0.03	0.69
Propionate	18.2	18.2	19.6	20.3	0.69	0.20	0.40	0.20
Butyrate	8.03	7.36	10.3	9.22	1.26	0.17	0.60	0.17
Isobutyrate	0.656	0.639	0.670	0.679	0.661	0.30	0.70	0.30
Valerate	1.12	1.09	1.08	1.04	0.19	0.28	0.81	0.28
Isovalerate	0.982	0.935	0.982	0.926	0.05	0.53	0.45	0.53
Total SCFA	81.7	80.8	83.7	81.0	1.23	0.16	0.16	0.16
Acetate: propionate	2.76	2.76	2.69	2.61	0.17	0.75	0.64	0.75
NH_3-N[3], mg/dL	12.0	14.2	13.1	13.7	0.85	0.36	0.48	0.36

[1]Values are least square means obtained from 4 cows

[2]Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = diet including a extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant ; linPRO-R ®); and LPF = diet including a extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant). All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK)

[5]NH_3-N = ammonia nitrogen

Table 4.6: Omasal nutrient flow and digestion of nutrients in the rumen of lactating Holstein cows fed different flaxseed supplements [1]

		Diet[2]					Contrasts: *P*-values		
Item		CTL	RAW	LPR	LPF	SEM	RAW vs. LPR	LPR vs. LPF	CTL vs. RAW+LPR+LPF
DM									
	Intake, kg/d	25.8	24.9	24.5	24.4	0.83	0.55	0.44	0.18
	Omasal flow, kg/d	15.2	15.9	15.5	14.4	1.36	0.84	0.41	0.97
	Apparent ruminal digestion, kg/d	9.04	7.48	10.9	11.0	1.15	0.04	0.04	0.37
	Apparent ruminal digestion, % of DMI	37.5	37.3	36.5	42.1	6.65	0.93	0.62	0.89
OM									
	Intake, kg/d	23.4	23.0	22.3	22.8	0.75	0.10	0.52	0.05
	Omasal flow, kg/d	13.5	14.0	13.8	12.8	1.23	0.88	0.46	0.95
	Apparent ruminal digestion, kg/d	8.74	7.84	10.6	10.2	0.99	0.03	0.05	0.25
	Apparent ruminal digestion, % of OMI	40.3	40.2	38.8	43.3	6.11	0.87	0.72	0.95
ADF									
	Intake, kg/d	4.86	5.60	4.82	4.79	0.38	0.22	0.21	0.66
	Omasal flow, kg/d	3.73	3.28	3.65	3.08	0.38	0.48	0.72	0.38
	Apparent ruminal digestion, kg/d	1.62	2.06	0.805	1.86	0.59	0.28	0.80	0.95
	Apparent ruminal digestion, % of ADFI	22.7	37.2	24.3	36.4	10.10	0.41	0.96	0.44
NDF									
	Intake, kg/d	7.49	7.39	7.65	7.49	0.46	0.30	0.90	0.99
	Omasal flow, kg/d	5.65	4.77	5.36	4.70	0.49	0.39	0.91	0.23
	Apparent ruminal digestion, kg/d	1.73	1.88	2.70	3.17	0.41	0.02	<0.01	<0.01
	Apparent ruminal digestion, % of NDFI	23.5	31.5	32.5	35.2	6.76	0.91	0.68	0.22

[1]Values are least square means obtained from 4 cows

[2]Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = diet including a extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant ; linPRO-R ®); and LPF = diet including a extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant). All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK)

unaffected by treatment. No differences in omasal flow of DM or OM were observed among dietary treatments. The apparent ruminal digestion of DM, when expressed as a percentage of DMI, was unaffected by dietary treatment; however, when expressed as kg/d, cows fed RAW diet had lower apparent ruminal digestibility of DM than those fed LPR diet (P = 0.04), and cows fed LPR diet had higher apparent ruminal digestibility of DM than those fed LPF diet (P = 0.04). A similar pattern was observed for the apparent ruminal digestibility of OM. The apparent ruminal digestibility of ADF (kg/d) was similar among treatments, whereas apparent ruminal digestibility of NDF (kg/d) improved in cows fed the RAW, LPR and LPF diets compared to those fed the CTL diet (P = <0.01). When expressed as a percentage of ADF and NDF intake, no differences in apparent ruminal digestibility were observed among treatments.

4.5 Omasal flow of nitrogen fractions and microbial protein

Nitrogen intake, omasal flow of N constituents and apparent ruminal digestibility of N constituents from the four ruminally-cannulated cows are presented in Table 4.7. No differences were observed in N intake or omasal flow of N when the CTL diet was compared to the average of all three flaxseed treatments. When comparing the apparent ruminal digestibility of N as a percentage of N intakes, no differences were reported between the CTL and the average of all three flaxseed treatments, nor where differences observed between the LPR fed cows to the RAW fed cows. Cows fed the LPF diet had higher apparent ruminal digestibility of N compared those fed the LPR diet (P = 0.06). Omasal flow of NH_3-N averaged 37.3 g/d, with no differences observed among treatments. Non-ammonia nitrogen (NAN) was unaffected by dietary treatment, and no differences were observed when NAN was corrected for microbial nitrogen (NANMN). Total microbial NAN flows were similar among treatments and averaged 452 g/d.

Table 4.7: Intake, digestibility and omasal flow of nitrogen (N) constituents in Holstein cows fed different flaxseed supplements [1]

	Diet[2]					Contrasts: *P*-values		
Item	CTL	RAW	LPR	LPF	SEM	RAW vs. LPR	LPR vs. LPF	CTL vs. RAW+LPR+LPF
N intake, g/d	657	650	649	641	12.6	0.67	0.99	0.52
N apparently digested in the rumen								
g/d	-38.6	-48.8	-93.8	-31.1	23.6	0.25	0.63	0.53
% of N intake	-6.02	-13.6	-14.6	-4.78	2.35	0.80	0.06	0.16
Flow at omasal canal								
N								
g/d	725	702	709	691	19.7	0.83	0.73	0.36
% of N intake	106	184	115	105	35.3	0.22	0.17	0.52
NH_3-N[4] g/d	36.7	31.2	43.3	38.1	7.16	0.21	0.46	0.90
NAN[5]								
g/d	686	672	664	655	13.8	0.72	0.49	0.23
% of N intake	103	104	104	101	3.00	0.93	0.50	0.88
NANMN[6]								
g/d	204	227	224	214	12.0	0.88	0.48	0.25
% of NAN flow	31.1	33.1	32.5	33.3	1.57	0.76	0.95	0.30
Total microbial NAN								
g/d	465	452	460	432	25.1	0.84	0.62	0.60
% of NAN	68.9	67.6	66.9	66.7	1.57	0.76	0.95	0.30

[1]Values are least square means obtained from 4 cows

[2]Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = diet including a extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant ; linPRO-R ®); and LPF = diet including a extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant). All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK)

[3]NH_3-N: ammonia nitrogen

[4]NAN: non-ammonia nitrogen

[5]NANMN: non-ammonia, non-microbial nitrogen

4.6 Intake and omasal flow of crude fat and individual fatty acids

Crude fat and fatty acid intakes from the four ruminally-cannulated cows are presented in Table 4.8. Crude fat intakes were higher when cows were fed the flaxseed treatments compared to when cows were fed the CTL treatment (P = <0.01). The intake of C14:0 was lower in the RAW, LPR and LPF treatments compared to the CTL treatment (P = 0.02). The intake of C18:0 was higher for cows fed the RAW, LPR and LPF diets compared to those fed the CTL diet (P = 0.02). The average intake of C18:2 n-6 in cows fed RAW, LPR and LPF diets was higher compared to cows fed the CTL diet (P = 0.01). When comparing the RAW and LPR treatments, intake of C18:2 n-6 was higher for the LPR-fed cows compared to RAW-fed cows (P = 0.04). Intake of C18:3 n-3 was higher in cows fed RAW, LPR and LPF diets compared to those fed the CTL diet (P = 0.02); however, when comparing flaxseed treatments, C18:3 n-3 intake was lower in cows fed the RAW diet compared to those fed the LPR diet (P = 0.04), and C18:3 n-3 intake was higher in cows fed the LPR diet compared to those fed the LPF diet (P = 0.05).

The omasal flow of crude fat and fatty acids from the four ruminally-cannulated cows are presented in Table 4.9. The average flow rate of C18:3 n-3 was increased when comparing the flaxseed treatments to the CTL treatment (P = 0.04). Furthermore, feeding the RAW diet resulted in higher omasal flows of C18:3 n-3 compared to feeding the LPR diet (P = 0.02). The omasal flow of C18:3 n-3 was higher in cows fed the LPF diet compared to those fed the LPR diet (P = 0.02). The omasal flows of C18:0 and C18:1 were not affected by dietary treatment. The omasal flow of total CLA isomers did not increase when the combined average of RAW, LPR and LPF treatments were compared to the CTL treatment (P = 0.82). Similarly, no differences were observed in omasal flow of total CLA between the RAW and LPR treatments (P = 0.24). Cows fed the LPF treatment had higher omasal flow of total CLA compared to cows fed the LPR

Table 4.8: Total crude fat and fatty acid intakes (g/d) of lactating Holstein cows fed different flaxseed supplements [1]

	Diet[2]					Contrasts: *P*-values		
Item	CTL	RAW	LPR	LPF	SEM	RAW vs. LPR	LPR vs. LPF	CTL vs. RAW+LPR+LPF
Crude fat	857	1490	1310	1320	37.8	<0.01	<0.01	<0.01
Total fatty acids	796	1120	1110	1160	52.4	0.91	0.65	0.02
C14:0	5.64	5.83	5.13	4.99	0.344	0.17	0.11	0.42
C14:1	0.145	0.099	0.114	0.118	0.012	0.42	0.32	0.05
C15:0	0.904	0.981	0.955	0.946	0.064	0.96	0.64	0.31
C16:0	221	229	224	215	11.7	0.78	0.42	0.90
C16:1	3.34	3.55	3.53	3.45	0.150	0.92	0.56	0.31
C18:0	40.5	45.1	55.5	51.5	2.68	0.04	0.15	0.02
C18:1	151	181	263	239	18.4	0.03	0.08	0.02
C18:2 n-6	272	304	333	365	15.8	0.04	0.24	0.02
C18:3 n-6	0.019	0.021	0.023	0.016	0.003	0.72	0.28	0.72
C18:3 n-3	186	261	433	442	45.3	0.04	0.05	0.02
C18:4 n-3	0.001	0.024	0.023	0.013	0.017	0.97	0.66	0.38
C20:0	2.72	3.04	3.34	3.45	0.154	0.11	0.22	0.03
C20:1	4.15	4.63	5.24	4.85	0.252	0.16	0.58	0.05
C20:2 n-6	0.15	0.041	0.173	0.229	0.025	<0.01	0.01	0.01
C20:3 n-6	0.004	0.004	0.016	0.019	0.010	0.27	0.21	0.62
C20:3 n-3	0.001	0.071	0.148	0.240	0.054	0.07	0.37	0.05
C20:4 n-3	0.000	0.000	0.001	0.001	0.018	0.24	0.22	0.58
C20:5 n-3	0.051	0.062	0.057	0.064	0.008	0.72	0.88	0.36
C22:0	3.10	3.37	3.86	3.63	0.160	0.06	0.25	0.03

Table 4.8 (cont'd): Total crude fat and fatty acid intakes (g/d) of lactating Holstein cows fed different flaxseed supplements [1]

	Diet[2]					Contrasts: *P*-values		
Item	CTL	RAW	LPR	LPF	SEM	RAW vs. LPR	LPR vs. LPF	CTL vs. RAW+LPR+LPF
C22:1	3.13	3.09	2.96	2.71	0.278	0.74	0.36	0.52
C22:2 n-6	0.00	0.00	0.001	0.002	0.009	0.86	0.40	0.62
C22:4 n-6	0.101	0.094	0.157	0.163	0.035	0.25	0.22	0.41
C22:5 n-6	0.001	0.001	0.001	0.001	0.000	0.55	0.29	0.67
C22:5 n-3	0.045	0.053	0.048	0.045	0.004	0.35	0.10	0.57
C22:6 n-3	0.039	0.045	0.054	0.051	0.009	0.44	0.61	0.30
C24:0	1.73	1.88	2.31	2.14	0.132	0.06	0.18	0.05
C24:1	0.428	0.410	0.391	0.441	0.038	0.71	0.53	0.79
Total SFA	277	276	296	273	16.1	0.46	0.89	0.82
Total MUFA	161	167	229	285	25.9	0.02	0.15	0.08
Total PUFA	457	566	812	765	61.3	0.04	0.08	0.02
Total PUFA n-3	158	264	448	428	46.1	0.04	0.05	0.01
Total PUFA n-6	265	306	361	332	18.0	0.08	0.34	0.03

[1]Values are least square means obtained from 4 cows

[2]Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = diet including a extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant ; linPRO-R ®); and LPF = diet including a extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant). All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK)

Table 4.9: Omasal flow of crude fat and fatty acids in lactating Holstein cows fed different flaxseed supplements [1]

	Diet[2]					Contrasts: *P*-values		
Item	CTL	RAW	LPR	LPF	SEM	RAW vs. LPR	LPR vs. LPF	CTL vs. RAW+LPR+LPF
Crude fat flow (g/d)	715	1049	1164	998	100	0.36	0.68	0.02
% Crude fat intake	85.0	70.2	88.9	75.5	9.50	0.17	0.69	0.56
Total Fatty acid flow (g/d)	663	970	1077	929	91.9	0.35	0.71	0.01
% Crude fat flow	92.5	92.6	92.6	93.2	0.223	0.94	0.11	0.38
Individual fatty acid flow (g/d)								
C14:0	17.7	11.9	10.2	9.88	1.49	0.48	0.40	<0.01
C14:1	3.47	2.70	2.12	2.00	0.276	0.13	0.18	0.05
C15:0	11.2	7.98	5.80	5.87	0.681	0.27	0.28	0.11
C16:0	468	325	288	269	30.8	0.40	0.23	<0.01
C16:1	0.083	1.23	0.923	1.31	0.084	0.03	0.44	<0.01
C18:0	411	538	523	458	51.9	0.81	0.26	0.12
C18:1	166	211	264	285	28.8	0.22	0.30	0.16
C18:2 n-6	1.24	1.34	2.69	3.18	0.302	0.02	0.01	0.02
CLA	4.58	2.63	4.29	6.06	0.913	0.24	0.03	0.82
cis-9, trans11	3.89	2.49	3.70	5.31	0.781	0.26	0.03	0.95
trans-10, cis-12	0.613	0.248	0.547	0.746	0.088	0.04	0.01	0.31
C18:3 n-3	7.63	56.9	14.0	14.8	2.51	0.02	0.02	0.04
C20:0	11.3	10.6	12.8	15.1	1.17	0.18	0.02	0.25
C20:1	2.29	1.62	1.65	2.38	0.342	0.94	0.11	0.28
C20:2 n-6	0.006	0.054	0.019	0.099	0.042	0.60	0.50	0.36
C20:3 n-6	0.048	0.049	0.205	0.366	0.018	0.12	0.06	0.10

Table 4.9 (cont'd): Omasal flow of crude fat and fatty acids in lactating Holstein cows fed different flaxseed supplements [1]

	Diet[2]					Contrasts: *P*-values		
Item	CTL	RAW	LPR	LPF	SEM	RAW vs. LPR	LPR vs. LPF	CTL vs. RAW+LPR+LPF
C20:4 n-6	0.00	0.088	0.007	0.057	0.029	0.14	0.49	0.25
C20:3 n-3	0.01	0.00	0.00	0.00	0.004	1.00	0.86	0.26
C20:5 n-3	0.00	0.00	0.00	0.02	0.015	0.86	0.40	0.62
C22:0	4.01	3.87	3.62	3.14	0.465	0.72	0.33	0.45
C22:1	3.37	2.18	2.09	1.64	0.332	0.83	0.23	<0.01
C24:0	4.15	3.62	3.00	3.10	0.405	0.34	0.42	0.11
C24:1	0.445	0.123	0.211	0.106	0.069	0.45	0.88	0.02
Total SFA[3]	769	783	994	909	71.7	0.18	0.33	0.30
Total MUFA[4]	176	220	292	270	28.4	0.22	0.31	0.16
Total PUFA[5]	56.4	112	56.9	60.3	5.29	<0.01	0.01	0.05
Total n-3[6]	7.65	56.8	14.0	14.8	2.50	0.01	0.01	0.03
Total n-6[7]	43.9	53.7	36.5	40.1	3.29	0.07	0.10	0.90

[1]Values are least square means obtained from 4 cows

[2]Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = diet including a extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant ; linPRO-R ®); and LPF = diet including a extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant). All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK)

[3]Total SFA = Total BCFA + C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C22:0 + C24:0 + C26:0

[4]Total MUFA = C14:1 + C16:1 + C18:1 + C20:1

[5]Total PUFA = Total n3 PUFA + Total n6 PUFA

[6]Total n3 PUFA = C18:3 n-3 + C20:3 n-3 + C20:5 n-3 + C22:3 n-3 + C22:5 n-3.

[7]Total n6 PUFA = C18:2 n-6 + C18:3 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:4 n-6.

treatment (P = 0.03), with a similar pattern observed for the *cis-9, trans-11* and *trans-10, cis-12* CLA isomers. Cows fed the flaxseed treatment diets had higher omasal flow rates of C18:2 n-6 compared to the CTL (P = 0.02). Cows fed the RAW treatment had lower omasal flows of C18:2 n-6 compared to those fed the LPR treatment (P = 0.02), and cows fed the LPF diet had higher omasal flows of C18:2 n-6 compared to those fed LPR diet (P = 0.01). Overall, total SFA and MUFA omasal flows were unaffected by dietary treatment; however, the flow rates of total PUFA were higher in cows fed the flaxseed treatments compared to those fed the CTL treatment (P = 0.05), higher in cows fed the RAW treatment compared to those fed the LPR diet (P = 0.01), and higher in cows fed the LPF treatment compared to those fed the LPR treatment (P = 0.01).

4.7 Dry Matter Intake, Milk Production, and Feed Efficiency

4.7.1 Dry matter intake

Results for DMI of all eight dairy cows are presented in Table 4.10. The average DMI for cows fed the RAW, LPR and LPF diets was lower than that for cows fed the CTL diet (P = 0.02). When DMI of cows fed the RAW diet was compared to that of cows fed the LPR treatment, it did not differ (P = 0.22). Additionally, no differences in DMI were observed when cows were fed the LPR diet compared to the LPF diet (P = 0.29).

4.7.2 Milk Production

Milk production data for all eight cows are presented in Table 4.10. A tendency for increased milk yield was observed when the combined average of RAW, LPR and LPF treatments was compared to the CTL diet (P = 0.07). Milk yield was lower in cows offered the RAW diet were compared to those offered the LPR diet (P =0.02) and no differences were observed when cows fed LPR diet were compared to those fed LPF diet (P = 0.46). However,

when expressed as ECM, no differences were observed among treatments and the overall ECM average from all eight cows was 40.5 kg/d.

4.7.3 Feed efficiency

The feed efficiency of all eight cows was calculated as ECM divided by DMI (Table 4.10). No differences were observed when comparing CTL diet to the combined average of RAW, LPR and LPF diets, nor when comparing the LPR treatment to the LPF. A tendency for improved feed efficiency was observed when cows fed the LPR diet were compared to those fed the RAW diet ($P = 0.08$).

4.8 Milk composition

Milk composition data collected from all eight dairy cows are presented in Table 4.10. Milk fat content decreased when the combined average of RAW-, LPR- and LPF-fed cows was compared to CTL-fed cows ($P = 0.03$). When the milk fat content of cows fed RAW versus LPR ($P = 0.14$) or LPR versus LPF ($P = 0.49$) were compared, no differences were observed. However, when milk fat is expressed as kg/d, no differences were observed among treatments. Milk protein content did not differ among treatments; however, a trend was observed for increased milk protein yield when the average of RAW, LPR and LPF diets was compared to the CTL diet ($P = 0.10$). Both lactose content and yield increased when the average of all RAW-, LPR- and LPF-fed cows was compared to the CTL-fed cows ($P = <0.01$). There was a trend for increased lactose content when LPR diet was compared to LPF diet ($P = 0.08$), as well as a trend for increased lactose yield when RAW diet was compared to LPR diet ($P = 0.09$). No differences were observed in the MUN when comparing the average of RAW-, LPR- and LPF-fed cows to CTL-fed cows ($P = 0.96$). However, there was a trend for lower MUN when cows

were fed LPR diet compared to RAW diet (P = 0.10). Somatic cell count (SCC) was unaffected by diet.

4.9 Milk fatty acid composition

The milk fatty acid compositional data from all eight cows are presented in Table 4.11. Milk SFA content was lower when the average SFA in the milk of the RAW-, LPR- and LPF- fed cows was compared to the milk of CTL-fed cows. (P = <0.01). The milk fatty acid contents of C10:0 (P = 0.05), C14:0 (P = <0.01), C15:0 (P = 0.02), C16:0 (P = <0.01) and C17:0 (P = <0.01) were lower in flaxseed-fed cows compared to CTL-fed cows. Milk C18:0 levels increased when the average of RAW, LPR and LPF treatments was compared to the CTL treatment (P = <0.01; Table 4.11). When comparing flaxseed supplements, no differences were observed in C18:0 milk fatty acid content between RAW and LPR (P = 0.876) or LPR and LPF (P = 0.35). Total milk MUFA was higher in the flaxseed treatments compared to the CTL treatment (P = <0.01). When comparing cows fed the RAW diet versus the LPR diet, total milk MUFAs increased in the LPR fed cows (P = <0.01). Cows fed RAW, LPR and LPF diets had increased levels of total milk PUFAs compared to those fed the CTL diet (P = 0.01), with concentrations of C18:2 n-6 and C18:3 n-3 showing the greatest change. The level of C18:2 n-6 was higher in cows fed the CTL diet compared to the average of those fed the RAW, LPR and LPF diets (P = 0.01). The levels of C18:3 n-3 in the milk of cows fed flaxseed treatments were higher compared to cows fed the CTL treatment (P = <0.01). When comparing C18:3 n-3 milk levels between flaxseed treatments, LPR had higher levels compared to RAW (P = <0.01) while no differences were observed between LPR and LPF. Milk fatty acid levels of C20:4n-3 (P = <0.01) and C20:5n-3 (P = <0.01) increased when the averages from RAW, LPR and LPF treatments were compared to the CTL treatment with no differences observed among flaxseed treatments. The n-

6: n-3 fatty acid ratio of milk decreased when cows were fed flaxseed treatments compared to the CTL treatment. Furthermore, the n-6: n-3 ratio was lower in the milk of cows fed LPR compared to those fed RAW (P = <0.01) while no differences were observed when comparing LPR and LPF treatments.

Table 4.10: Dry matter intake (DMI), milk yield and milk composition of lactating Holstein cows fed different flaxseed supplements[1]

	Diets[2]					Contrasts: *P*-values		
Item	CTL	RAW	LPR	LPF	SEM	RAW vs. LPR	LPR vs. LPF	CTL vs. RAW+LPR+LPF
DMI, kg/d	25.9	24.4	23.4	24.2	0.460	0.22	0.29	0.01
Body Weight (kg)	722	716	716	717	21.5	0.99	0.90	0.39
Body Weight Change (kg/d)	0.203	0.078	0.237	0.393	0.176	0.57	0.59	0.89
Milk Yield, kg/d	41.9	42.3	44.4	43.8	1.23	0.02	0.46	0.07
ECM[3], kg/d	39.9	40.2	41.1	40.9	1.46	0.56	0.87	0.48
Feed Efficiency[4]	1.61	1.68	1.71	1.74	0.066	0.08	0.54	0.30
Milk Fat								
%	3.49	3.39	3.15	3.04	0.130	0.14	0.49	0.03
kg/d	1.38	1.42	1.36	1.34	0.070	0.45	0.85	0.94
Milk Protein								
%	3.12	3.08	3.00	3.05	0.060	0.15	0.37	0.14
kg/d	1.26	1.28	1.31	1.34	0.030	0.37	0.28	0.10
Milk Lactose								
%	4.45	4.51	4.51	4.56	0.040	0.91	0.08	<0.01
kg/d	1.81	1.85	1.95	1.98	0.060	0.09	0.56	0.03
Milk Urea Nitrogen, mg/dL	11.2	11.8	10.3	11.3	0.680	0.10	0.25	0.96
Somatic Cell Count, x 1,000 cells/mL	86.5	96.5	90.1	81.4	13.4	0.27	0.14	0.59

[1]Values are least square means obtained from 8 cows

[2] Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = diet including a extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant ; linPRO-R ®); and LPF = diet including a extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant). All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK)

[3]Energy-corrected milk (ECM), [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)] (Orth, 1992).

[4]Feed Efficiency: ECM/DMI

Table 4.11: Milk fatty acid profile (% of FAME) in milk of lactating Holstein cows fed different flaxseed supplements[1]

	Diet[2]					Contrasts: *P*-values		
Item	CTL	RAW	LPR	LPF	SEM	RAW vs. LPR	LPR vs. LPF	CTL vs. RAW+LPR+LPF
C4:0	0.584	0.639	0.611	0.594	0.028	0.37	0.53	0.24
C6:0	1.25	1.39	1.19	1.35	0.106	0.21	0.30	0.63
C10:0	2.47	2.36	2.02	1.95	0.150	0.03	0.16	0.05
C12:0	3.38	3.09	2.55	2.38	0.128	<0.01	0.23	<0.01
C14:0	11.8	11.3	9.49	9.68	0.250	<0.01	0.48	<0.01
C15:0	1.18	1.07	0.902	0.924	0.057	0.48	0.26	0.02
C16:0	42.2	34.3	29.3	29.7	0.722	<0.01	0.52	<0.01
C16:1	1.93	1.37	1.35	1.55	0.207	0.89	0.24	<0.01
C17:0	0.494	0.459	0.415	0.407	0.008	<0.01	0.11	<0.01
C18:0	8.04	12.1	12.2	11.6	0.869	0.88	0.35	<0.01
C18:1	20.3	25.8	33.1	32.3	0.726	<0.01	0.30	<0.01
C18:2 n-6	2.37	2.12	2.10	2.06	0.055	0.84	0.64	<0.01
CLA[4]	0.281	0.308	0.845	0.841	0.078	<0.01	0.96	<0.01
cis-9, trans-11	0.240	0.266	0.696	0.698	0.069	<0.01	0.99	<0.01
trans-10, cis-12	0.027	0.029	0.100	0.094	0.009	<0.01	0.65	<0.01
C18:3 n-6	0.049	0.068	0.092	0.129	0.013	0.19	0.05	<0.01
C18:3 n-3	0.433	0.745	0.950	0.981	0.031	<0.01	0.53	<0.01
C20:0	0.160	0.166	0.283	0.251	0.050	<0.10	0.65	0.20
C20:1	0.375	0.345	0.514	0.560	0.043	0.01	0.38	0.04
C20:2 n-6	0.006	0.009	0.007	0.006	0.003	0.63	0.84	0.60
C20:3 n-6	0.113	0.107	0.072	0.079	0.006	<0.01	0.29	<0.01

Table 4.11(Cont'd): Milk fatty acid profile (% of FAME) in milk of lactating Holstein cows fed different flaxseed supplements[1]

	Diet[2]					Contrasts: *P*-values		
Item	CTL	RAW	LPR	LPF	SEM	RAW vs. LPR	LPR vs. LPF	CTL vs. RAW+LPR+LPF
C20:3 n-3	0.001	0.000	0.000	0.000	0.001	0.23	0.27	0.62
C20:4 n-3	0.013	0.039	0.046	0.050	0.004	0.12	0.22	<0.01
C20:5 n-3	0.056	0.071	0.074	0.072	0.004	0.55	0.70	<0.01
C22:0	0.045	0.054	0.048	0.046	0.005	0.45	0.81	0.51
C22:1	0.050	0.040	0.030	0.038	0.007	0.49	0.36	0.12
C22:4 n-6	0.000	0.001	0.007	0.003	0.003	0.16	0.68	0.31
C22:5 n-3	0.076	0.084	0.074	0.078	0.006	0.12	0.55	0.68
C22:6 n-3	0.000	0.000	0.000	0.000	0.001	0.18	0.99	0.57
C24:0	0.028	0.061	0.028	0.040	0.009	0.02	0.34	0.15
C24:1	0.000	0.000	0.000	0.000	0.001	0.97	0.95	0.16
Total SFA[3]	72.7	67.8	60.2	57.7	0.812	<0.01	0.63	<0.01
Total MUFA[4]	23.7	28.5	36.0	35.4	0.785	<0.01	0.57	<0.01
Total PUFA[5]	3.55	3.71	4.35	4.38	0.142	<0.01	0.84	<0.01
Total n-3 PUFA[6]	0.579	0.940	1.15	1.18	0.035	<0.01	0.52	<0.01
Total n-6 PUFA[7]	2.68	2.45	2.38	2.36	0.070	0.51	0.87	<0.01
n-6:n-3	4.67	2.64	2.06	2.01	0.088	<0.01	0.72	<0.01

[1]Values are least square means obtained from 8 cows

[2]Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement; LPR = diet including a extruded flaxseed and pea supplement; and LPF = diet including a extruded flaxseed and high tannin faba bean supplement. All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK)

[3]Total SFA = C10:0 + C12:0 + C13:0 +C14:0 +C15:0 + C16:0 +C17:0 + C18:0 + C19:0 + C20:0 + C22:0 + C24:0+C26:0

[4]Total MUFA = C14:1 + C16:1 + C18:1 + C20:1.

[5]Total PUFA = Total n-3 PUFA + Total n-6 PUFA

[6]Total n3 PUFA = C18:3 n-3 + C20:3 n-3 + C20:5n-3 + C22:3 n-3 + C22:5 n-3.

[7]Total n6 PUFA = C18:2 n-6 + C18:3 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:4 n-6.

5.0 DISCUSSION

In the present study, flaxseed supplements were formulated to have similar ingredient compositions and to vary only in processing or the inclusion of condensed tannins. Even though ingredient compositions of the flaxseed ingredients were similar, the EE content was lower in the LPR and LPF supplements (averaging 22.4% DM) compared to the RAW supplement at 27.5% DM. These discrepancies in EE contents of flaxseed supplements could be attributed to the occurrence of oil leaching which has previously been identified as a consequence to extrusion processing (Iram et al., 2012). Condensed tannins were higher in the LPF supplement compared to the LPR supplement, as was expected. However, condensed tannin content of the LPF supplement decreased dramatically from 6.87 mg/g prior to extrusion to 1.17 mg/g after extrusion. These observations support the findings of Imran et al. (2012), who reported a 42.9% to 77% reduction in tannin compounds when flaxseed meal was extruded. The high temperatures (greater than 110 °C) are the main cause for reduced tannin compounds in extruded feed products (Iram et al., 2012). Moreover, the rate at which ingredients are fed into the extruder may also play a role in degree of tannin compound reduction in extruded supplements. According to Iram et al. (2012) tannin levels in extruded products may also be influenced by the rotation speed of the screw and the rate at which ingredients are fed into the extruder. The present study did not record the rotation speed of the extruder screw nor was ingredient feeding rate recorded; therefore, it may prove beneficial to evaluate different extrusion processing techniques to minimize the loss of condensed tannins in extruded flaxseed supplements designed for use in ruminant diets.

Feeding high fat diets (greater than 6 % DM) to dairy cattle has been suggested to impair ruminal fibre digestion and DMI, especially if the fat source is high in PUFA (NRC, 2001).

Furthermore, DMI in dairy cattle may also be limited by the inclusion of dietary condensed tannins (Patra and Saxena, 2011). In the present study, including the RAW, LPR and LPF flaxseed supplements in the experimental diets increased dietary EE content from 3.21 % to 5.52 % (DM basis). Due to the high EE levels of the diets containing flaxseed supplements, it is not surprising that DMI was decreased by 1.19 kg/d in cows fed the flaxseed supplements when compared to those fed the CTL diet. Typically, DMI has been reported to decrease when a fat source replaces a carbohydrate source in a cow's diet (NRC, 2001). Dietary fat sources have been shown to decrease ruminal digestibility of fibre (Palmquist and Jenkins, 1980; Chapula et al., 1986; Allen, 2000) which could contribute to distension of the rumen resulting in lower DMI (NRC, 2001). However, this study saw no differences in the apparent digestibility of ADF and NDF when cows were fed the RAW, LPR or LPF treatments compared to the CTL. These findings are supported by Ueda et al. (2003) who reported no effect on fibre digestibility in the rumen when dairy cows were supplemented with flaxseed oil at 3 % DM. This demonstrates that DMI was not limited by fibre digestibility in the present study. Instead, the observed decrease in DMI may be related to the effects of dietary PUFA on gut hormones. Litherland et al. (2005) found that DMI was decreased when up to 600 g of UFA from soy oil was infused into the abomasum of dairy cow daily for a period of 5 days. This was accompanied by an increase in plasma glucagon-like peptide-1 (**GLP-1**). Benson and Reynolds (2001) had previously identified GLP-1 as an intake suppressant in dairy cattle when vegetable oil was infused at the abomasum. In both the Litherland et al. (2005) study and the Benson and Reynolds (2001) study, the main UFA infused at the abomasum was C18:2 n-6. In the present study, an increase in the omasal flow rates of C18:2 n-6 from 1.24 g/d to 2.40 g/d was observed when comparing the CTL treatment to the combined average of the RAW, LPR and LPF treatments, respectively.

Therefore, it is tempting to speculate that the decrease in DMI when cows were fed a flaxseed supplement could be partly attributed elevations in GLP-1 due to increase flow of C18:2 n-6 from the rumen. When the LPR treatment was compared the LPF treatment, no differences in DMI were observed. In contrast, Dschaak et al. (2011) reported lower DMI when animals were fed condensed tannin extract at 3% of dietary DM. Similar results were reported by Priolo et al. (2000) when condensed tannins were included in the diet of sheep at 2.5 % of DM. Similarly, Carulla et al. (2005) reported a decrease in sheep DMI when 41 g/kg DM of *Acacia mearnsil* extract (containing 0.615 g of condensed tannins) was included in the diet. Based on these findings, the effect of condensed tannins on DMI may be dependent on the level of dietary inclusion or the source of condensed tannins. It is possible that the inclusion level of condensed tannins in the present study was too low to observe a response in DMI when compared to LPR.

In the present study, it was observed that supplementing a dairy cow's diet with flaxseed tended to increase milk yield when compared to the control diet. Milk yield increased from 41.9kg/d in the CTL treatments to 43.5 kg/d in the combined average of RAW, LPR and LPF treatments. The increase in milk yield observed in the present study may be related to the elevated levels of dietary energy in the flaxseed treatments (averaging 1.44 Mcal/kg) compared to the CTL treatment (1.35 Mcal/kg). Milk yield increased by 2.1 kg/d when the RAW treatment was compared to the LPR treatment. According to Petit (2010), physical processing of flaxseed typically results in increase milk yields; however, the mechanism(s) responsible for such a response remain unclear. Whole flaxseed has been reported to have poor total tract digestibility (Martin et al., 2008); therefore, it is possible that the lower milk yield observed in the RAW treatment versus the LPR treatment is due to poor digestibility of nutrients and, therefore, less available energy for milk production. Because milk yield increased in the LPR-fed cows

compared to the RAW while simultaneously maintaining similar DMI, the feed efficiency of LPR-fed cows was improved from 1.68 to 1.71, respectively. In general, results of this trial demonstrated that RAW, LPR and LPF flaxseed supplements can be safely included in dairy rations at 11.4% of DM without negatively impacting animal performance.

Although marker dysfunction with the triple marker technique necessitated the use of a single marker (iNDF) to estimate omasal digesta flow, the omasal flows of DM and OM that are reported in the present study fall within the biological limits reported previously (Titgemeyer, 1997) and therefore validate the omasal nutrient flow data reported in this study (Ahvenjairi et al., 2000). In the present study, estimates of DM and OM flow at the omasal canal were within a range of 14.4 to 15.9 kg/d and 12.8 to 14.0 kg/d, respectively, with no differences observed among treatments. These estimates are higher than those reported by Sterk et al. (2012) who used the triple-marker technique to evaluate nutrient flow of animals fed differed flaxseed products. Chibisa et al. (2012) also used the triple-marker technique to evaluate nutrient flows of animals fed different dietary inclusions of distiller's grains and reported higher flow rates of DM and OM in the range of 22.3 kg/d to 24.2 kg/d and 18.0 kg/d to 19.5 kg/d, respectively. The differences in flow rates reported in the present study compared to Sterk et al. (2012) and Chibisa et al. (2012) may be due to differences in DMI. According to Brito et al. (2006), higher DMI is often associated with higher omasal flow of DM and OM. In the current study DMI averaged 24.9 kg/d compared to the 20.6 kg/d DMI reported by Sterk et al. (2012) and the 30.2 kg/d DMI reported by Chibisa et al. (2012). Results of this study showed no difference in the apparent ruminal digestibility of DM or OM when expressed as a percentage of DMI and OMI. When expressed as a percentage of nutrient intake, the apparent digestibility of DM, OM, and fibre fractions were unaffected when cows were supplemented with RAW, LPR or LPF compared to the CTL in the

current study. This is consistent with the nutrient digestibility data reported by Ueda et al. (2003) when flaxseed oil was supplemented at 3% DM.

In the present study, N intake and omasal flow of N were similar among treatments. These observations are supported by Chibisa et al. (2012), Brito et al. (2009) and Brito et al. (2007) who observed that omasal flow of N and N intake often parallel one another. The apparent ruminal digestion of N was unaffected by flaxseed supplementation when compared to CTL and averaged -62.7 g/d. The negative values reported for ruminal digestion of N indicate greater N flow compared to N intake. Similar findings have been reported throughout the literature (Chibisa et al., 2012; Olmos Colmenero and Broderick, 2006). The increase in N flow compared to N intake can be attributed to urea-N recycling from the bloodstream into the rumen (Broderick et al., 2008). When expressed as a percentage of N intakes, the apparent ruminal digestion of N was higher in the LPF treatment compared to the LPR. In the present study, it had been anticipated that the dietary inclusion of faba beans as a source of condensed tannins would limit ruminal digestion of N since condensed tannins have been found to complex with dietary protein, thereby limiting protein degradation by rumen microbes (McSweeney et al., 2001; Patra and Saxena, 2011). However, Perex-Malsonado et al. (1995) indicated that tannins differ in their ability to form strong complexes at normal ruminal pH. Therefore, it is possible that the condensed tannins in the present study formed a weak protein-tannin complex resulting in poor ruminal protection of dietary proteins. Furthermore, feeding the LPR supplement resulted in similar apparent ruminal digestion of N when compared to RAW supplement even though extrusion processing has been suggested to limit protein digestion in the rumen (Poncet et al., 2003). It is possible that the temperature used for extrusion in this trial (120°C) may not have been high enough to provide protection of dietary protein from ruminal digestion (Ueda et al.,

2003). However, Gonthier et al. (2004) also reported limited protection of extruded canola from ruminal CP degradation even when a higher temperature (155°C) was used during the extrusion process. Doreau et al. (2009) suggested that the oil content of extruded products may reduce the effectiveness of extrusion processing in protecting proteins, even if the oilseeds are extruded with absorbent materials.

The effect of lipid supplementation on NH_3-N flow is variable throughout the literature. In the present study, omasal flow of NH_3-N averaged 37.3 g/d with no differences observed among treatments. In contrast, Ueda et al. (2003) reported an increase in NH_3-N flow when cows were supplemented with flaxseed oil at 3% of DM, while other studies reported a decrease in NH_3-N flow when flaxseed was included in a cow's diet (Ikwuegbu and Sutton, 1982; Broudiscou et al., 1994). A review by Doreau and Ferlay (1995) concluded that lipid supplementation is not responsible for variations in NH_3-N outflow; instead, these changes are believed to be associated with altered rumen protein degradation or rumen protein synthesis. The omasal flow of NH_3-N is dependent on ruminal NH_3-N concentration (Brito and Broderick, 2007); in the present study, ruminal NH_3-N concentrations were similar in cows fed the RAW, LPR and LPF diets, so the lack of treatment differences in omasal NH_3-N flow among these treatments is not surprising.

Including PUFA in the diets of ruminants may have a toxic effect on certain ruminal microorganisms, including the protozoal population, which could decrease protozoal predation on bacteria thereby promoting bacterial proliferation (Jouany and Ushida, 1999). Therefore, decreasing protozoal populations within the rumen may increase bacterial synthesis and increase omasal flow of microbial NAN. The flow of microbial NAN reported in other studies using ^{15}N as a microbial marker in addition to using the omasal sampling technique under various dietary

conditions range from 375 to 743 g/d (Brito et al., 2006; Brito et al., 2007; Chibisa et al., 2012; Olmos Colmenero and Broderick, 2006). Ueda et al. (2003) estimated microbial nitrogen yield using purine and pyrimidine bases with HPLC and reported microbial NAN flows within a range of 260 to 302 g/d when comparing diets supplemented with flaxseed oil to a diet that had no flaxseed oil. In the present study, total microbial NAN flows were within the above-mentioned range and were similar among treatments, averaging 351 g/d. Similar results were reported by Doreau et al. (2009) when comparing cows fed a control diet to those supplemented with rolled flaxseed, extruded flaxseed or flaxseed oil at 7.5% of DMI. Conversely, an earlier study by Broudiscou et al., (1994) reported increases in microbial nitrogen flow when UFA sources were fed to cattle.

Results of this study show no difference in milk protein content among treatments which is reflective of the similar N flow rates. These findings are supported by Petit and Gragnon (2009) who reported similar milk protein content and yield in mid-lactating dairy cattle fed between 5 and 15% whole flaxseed. Additionally, Gonthier et al (2005) reported similar milk protein concentrations when animals were fed no flaxseed compared to those fed grown, extruded or micronized flaxseed. No differences in MUN were observed between LPR and LPF treatments, nor did flaxseed supplementation have an effect when compared to CTL; however, there was a trend for lower MUN when animals were fed LPR compared to RAW. Sterk et al. (2014) reported no difference in MUN when different flaxseed treatments were fed to early lactating cows. Similarly, Neveu et al. (2013) saw no effect of MUN when comparing a control treatment with no flaxseed to an extruded flaxseed treatment. Excess dietary protein may result in increased production of NH_3-N in the rumen which is later converted to urea in the cow's liver and incorporated into the milk (Aguilar et al., 2012). Heat treatments, such as extrusion may

limit microbial availability of protein and potentially prevent accumulation of NH_3-N within the rumen (Petit, 2010). However, results of this study reported no difference in ruminal fluid NH_3-N concentrations or in the omasal flow rates of NH_3-N when RAW was compared to LPR. Therefore, an explanation for the tendency for LPR treatments to have lower MUN compared to the RAW treatment remains unclear.

The Canadian milk marketing system offers a premium for milk fat production. Increasing the content of PUFA in the milk may increase the risk for MFD and, therefore, could have a negative economic impact for producers. Results of this trial demonstrate a decrease in milk fat percentage by 0.30% units when the combined average of RAW, LPR and LPF treatments were compared to the CTL. Martin et al. (2008) reported a decrease in milk fat percentage from 4.11% to 3.53% when animals were fed diets containing 14.8% extruded flaxseed. Similarly, Neveu at al. (2013) reported a decrease in milk fat percentage when animals were fed a control diet with no flaxseed or a diet supplemented with 9.9% extruded flaxseed. A decrease in milk fat content has often been associated with an increase in mammary uptake of the *trans-10, cis-12* CLA isomer which has been associated with the down-regulation of key lipogenic enzymes in the mammary gland (Baumgard et al., 2000; Bauman and Griinari, 2003; Piperova et al., 2000). In the present study, the milk from cows fed flaxseed supplements had the highest milk fat content of the *trans-10, cis-12* CLA isomer which could potentially explain the lower milk fat content observed in cows fed these diets. However, when milk fat is expressed as yield (kg/d) no differences were observed among dietary treatments. This is in agreement with the findings of Gonthier et al. (2005) who reported similar milk fat yields between cows fed a control diet with no flaxseed and cows supplemented with 10% raw, micronized or extruded flaxseed. Since milk producers would receive payment based on milk fat yield and not milk fat

percentage, it is unlikely that feeding whole or extruded flaxseed products would negatively impact their economic return for this specific milk component. To demonstrate the economic value of the experiment diets used in this study, the income over feed costs were determined for using 2015 Saskatchewan feed costs and milk component prices (Appendix D). Also, feeding flaxseed supplements would not have a negative impact on producers' ability to meet their quota obligations as quota is based on milk fat yield.

The composition of bovine milk fat is greatly influenced by the production of SCFA through ruminal fermentation as well as the omasal flow of fatty acids. Therefore, a major objective of this study was to compare the effectiveness of the RAW, LPR and LPF flaxseed supplements on omasal flow or fatty acids and milk fatty acid composition. It was expected that supplementing dairy diets with LPR would be more effective than RAW at increasing omasal flows of PUFA and CLA isomers, subsequently resulting in increased milk fat content of these two fatty acid groups. Moreover, it was expected that feeding LPF compared to LPR would have an additive effect for increasing omasal flow and milk fat content of these desirable fatty acids that have been demonstrated to have beneficial effects on human health.

De novo synthesis of short- and medium-chain fatty acids (C4:0 to C14:0) in the mammary gland of dairy cattle is highly dependent upon ruminal fermentation end products. Microbial fermentation of dietary feed ingredients results in the formation of SCFA such as acetate, butyrate and propionate, all of which play a key role in the synthesis and formation of milk fat. Therefore, one of the objectives for this study was to investigate the effects of different flaxseed supplements on ruminal fermentation characteristics. In the present study, ruminal fluid concentrations of propionate and butyrate were unaffected by dietary treatment; however, acetate concentrations were 6.7 % lower in the ruminal fluid of cows fed the LPF diet compared to those

fed the LPR diet. These findings support those of Beauchemin et al. (2007) who reported a decrease in acetate concentrations when quebracho tannin extracts was included in the diet of Angus heifers and steers. In contrast, Tan et al. (2011) reported no effect on acetate production when condensed tannins were incubated in buffered rumen fluid *in vitro*. Because acetate is a common by-product of ruminal fibre digestion, decreased ruminal digestibility of fibre may result in lower ruminal fluid acetate concentrations. However, in the present study ruminal fibre (NDF) digestibility was actually higher in cows fed the LPF diet compared to those fed the LPR diet, which is inconsistent with the lower ruminal acetate concentrations that were observed in cows fed the LPF diet compared to those fed the LPR diet. The reasons for these discrepant results are unclear; however, it should be noted that ruminal SCFA concentrations might not be reflective of total SCFA production in the rumen. Despite the lower ruminal fluid concentrations of acetate reported in the LPF treatment versus the LPR treatment, no differences in C4:0 to C14:0 fatty acid concentrations were observed in the milk when comparing these two treatments.

Milk fatty acids with a carbon chain length of 16 or greater originate from mammary uptake of circulating fatty acids. Circulating fatty acids primarily originate from intestinal absorption of fatty acids leaving the rumen. According to Sterk et al. (2012), the flow of fatty acids from the rumen may be impacted by several factors, one of which is fatty acid intake by the animal. In the present study, intake of C18:3 n-3 in cows fed the RAW diet was lower compared to those fed the LPR diet. The differences in the C18:3 n-3 intakes may be related to the differences in dietary C18:3 n-3 levels. The RAW dietary treatment had a C18:3 n-3 content of 18.8% FAME while the LPR diet had a C18:3 n-3 content of 19.7 % FAME. The DMI was similar in cows fed the RAW and LPR diets, the differences in C18:3 n-3 intakes reflect the greater C18:3 n-3 concentrations in the LPR diet compared to the RAW diet. In the present

study, the omasal flow of EE and total fatty acids were lower that EE and total fatty acid intakes across all diets. Lower ruminal outflows of fatty acids compared to fatty acid intakes have also been reported by other workers (Chibisa et al., 2013; Glasser et al., 2008). A review by Jenkins (1993) indicates that fatty acid disappearance in the rumen is more common in diets supplemented with a fat source. The potential explanation for this is through microbial degradation of dietary fats to shorter fatty acid chain lengths, absorption of the shorter chain fatty acids through the rumen wall, or the underestimation of nutrient flow at the omasum (Wu et al., 1991).

If dietary PUFA are not protected from the ruminal microbial ecosystem, they will undergo extensive biohydrogenation and limit the amount of PUFA flow from the rumen for later incorporation into the milk. In the present study, cows fed RAW, LPR or LPF diets resulted in an increase in C18:3 n-3 flow from the rumen compared to the CTL diet. This then translated into a 106 % increase in milk content of C18:3 n-3 in cows fed the flaxseed-supplemented diets compared to the CTL. When comparing flaxseed treatments, feeding RAW was more effective than LPR at protecting C18:3 n-3 from microbial biohydrogenation resulting in higher omasal flow of C18:3 n-3 (+42 g/d) in cows fed the RAW diet compared to those fed the LPR diet. However, the increased omasal flow of C18:3 n-3 in the RAW treatment did not result in greater milk content of C18:3 n-3. Other studies have reported low total tract digestibility of whole flaxseed (Martin et al., 2008; Petit et al., 2005), which would limit C18:3 n-3 absorption from the small intestine, thus limiting its availability for mammary incorporation into milk fat. When cows were supplemented with LPF compared to LPR, the omasal flow of C18:3 n-3 also increased; however no differences were observed between the two diets in the milk fat content of C18:3 n-3. The lack of response observed in the milk of LPF-fed cows compared to LPR-fed

cows, despite LPF having higher flow rates of C18:3 n-3 remains unclear. It can be speculated that the LPF supplement has poorer total tract digestibility compared to the LPR supplement which would results in lower transfer efficiency of C18:3 n-3 from the small intestine to the mammary gland. Further research evaluating the total tract digestibility of the RAW, LPR and LPF supplements used in this study may provide more insight into these findings. The milk fat content of C18:2 n-6 was 11.8 % lower in the flaxseed treatments compared to the CTL, although the omasal flow of C18:2 n-6 was greater in cows fed flaxseed-supplemented diets compared to those fed the CTL diet. The higher C18:2 n-6 fatty acid content and lower C18:3 n-3 fatty acid content of milk from CTL-fed cows resulted in a higher n-6: n-3 ratio compared to the milk of cows fed flaxseed supplements. Furthermore, feeding LPR over RAW was more effective at lowering this ratio. Having a low n-6: n-3 ratio is suggested to be favorable for human health (Simopolous, 2006).

Microbial biohydrogenation of dietary PUFA is associated with an accumulation of biohydrogenation intermediates, such as C18:1 and CLA isomers, as well as C18:0 which is the end product of biohydrogenation. In the present study, no differences were observed in the omasal flows of C18:1 and C18:0 among treatments. These results are indicative of incomplete biohydrogenation of dietary PUFA since no accumulation of C18:1 or C18:0 were observed when cows were fed diets containing a flaxseed supplement. However, the milk fat content of both C18:0 and C18:1 increased when flaxseed was included in the diet of dairy cows. The omasal flow of CLA isomers did not increase when the three flaxseed treatments were compared to the CTL. However, a 41 % increase in omasal flow of CLA was observed when dairy cow diets were supplemented with LPF compared to LPR. *In vitro* trials have demonstrated the ability of condensed tannins to inhibit the final stages of biohydrogenation, resulting in an accumulation

of CLA isomers (Kronberg et al., 2007). However, when comparing the fatty acid profiles of the milk from cows fed either LPR or LPF, no differences in CLA isomer content was observed. The CLA content of bovine milk did increase by 137 % when the mean CLA content of all flaxseed treatments was compared the CTL. Moreover, feeding LPR resulted in a 64 % increase of milk CLA content compared to the RAW treatment. An increase in the *trans-10, cis-12* CLA isomer was observed when dairy cows were fed flaxseed supplements compared to the CTL. Increased mammary uptake of *trans-10, cis-12* CLA may also explain the lower levels of short- and medium-chain fatty acids in milk as discussed earlier. Piperova et al. (2000) observed a 40-60% reduction in mRNA encoding for the acetyl-CoA enzyme, which is necessary for *de novo* synthesis of medium-chain fatty acids in the mammary gland, when *trans-10, cis-12* CLA isomer was present. The formation of *trans-10, cis-12* CLA has been said to be a preferred biohydrogenation pathway when ruminal pH declines (Peterson et al., 2002). However, the present study saw no differences in ruminal fluid pH in cows fed RAW, LPR and LPF diets compared to the CTL. It is likely that the higher levels of *trans-10, cis*-12 CLA observed in the milk of fed a flaxseed-supplemented diet is attributed to the greater availability of C18:3n3 as a substrate for ruminal biohydrogenation compared to the CTL-fed cows. The milk fat content of the *cis-9, trans-11* CLA isomer was higher in the milk when cows were fed the extruded flaxseed product compared to the whole flaxseed product. Because the *cis-9,trans-11* CLA isomer has been associated with many human health benefits (Schwingshackl and Hoffman , 2012), feeding extruded flaxseed supplements is a more effective strategy for increasing levels of this desirable fatty acid in milk for human consumption.

Milk lactose content and yield increased when cows were supplemented with flaxseed products compared to the CTL. Additionally, a trend for increasing milk lactose content when

LPR was fed compared to LPF and when RAW was compared to LPR was observed. These results support the findings of Neveu et al. (2013), who reported an increase in milk lactose content when animals were supplemented with extruded flaxseed at 9.9 % of dietary DM. On the other hand, Sterk et al. (2014) reported no difference in milk lactose content when early lactating dairy cattle were supplemented with either extruded flaxseed, formaldehyde-treated flaxseed, or flaxseed and DHA oil. Furthermore, Petit et al. (2005) observed a decrease in milk lactose when whole flaxseed was fed to mid-lactating dairy cows at 11.8% of dietary DM compared to cows fed a control diet with no flaxseed supplementation. Mashek and Grummer (2003) indicated that rates of gluconeogenesis may increase when C18:3 n-3 is present. Moreover, March et al. (2013) found that feeding extruded flaxseed to dairy cattle increases expression of liver glucose-transporter-2 which could result in increased glucose secretion from the liver. Increased availability of glucose for lactose synthesis in the mammary gland could explain the observed increase in milk lactose content when flaxseed treatments were compared to the CTL (Petit et al., 2015). The present study did not look at the effects of RAW, LPR or LPF supplements on gene expression or gluconeogenesis but may be a promising area of research.

Increased somatic cell count (**SCC)** is milk is an indicator of inflammation within the cow's udder and is primarily caused by a bacterial infection (Dohoo and Meek, 1982). Animals with higher SCC tend to produce less milk which can have economic implications to the producers and negatively impact milk quality for consumers (Shock et al., 2015). According to Lessard et al. (2003), omega-3 fatty acids may alter the production of immunocompetent cells which may reduce the risk of infection. Therefore, feeding flaxseed supplements to dairy cattle would be expected to reduce milk SCC. In the present study, milk SCC was similar among treatments and no differences were observed between flaxseed supplements. These findings are

consistent with other studies in which flaxseed supplements were included in dairy diets with no beneficial impact on milk SCC (Petit, 2010; Sterk et al., 2012; Neveu et al., 2013).

6.0 GENERAL SUMMARY

Dairy products represent a major dietary component in North America which has led to an increase interest in the role milk fat on human health. Current research suggests that an ideal milk fat would contain increased levels of omega-3 PUFA and CLA with lower levels of SFA. Feeding a flaxseed supplement to dairy cattle has the potential to achieve these goals; however, it is important to consider the impact these supplements may have on ruminal fermentation and the potential consequences for animal performance and the economic return to producers. The purpose of this thesis research was to evaluate the effects of different flaxseed supplements on rumen fermentation, omasal flow of nutrients, milk fatty acid composition and animal performance.

Bovine milk is a common component in the North American diet and represents a major source of caloric intake (Zaripheh and Miller, 2008). As such, there is particular interest in the fatty acid composition of dairy products and its potential impact on human health. Many of the SFA found in bovine milk fat have a neutral impact on human health; however, there is continued concern for the levels of C12:0, C14:0 and C16:0 fatty acids since these have been associated with increased risk of inflammatory diseases (Calder, 2015). Furthermore, there is continued interest in the roles of PUFA on prevention of chronic diseases in humans. More specifically, there is a growing body of evidence related to the important role that the omega-3 fatty acids C18:3 n-3, C20:5 n-3 and C22:6 n-3 play in the regulating inflammatory illnesses, preventing certain forms of cancer, and treating neurological disorders (Calder, 2015; Ferruci et al., 2006; Gerber, 2012; Joshi et al., 2006; Makarem et al., 2013). Bovine milk fat also contains the unique group of *trans* fatty acids known as CLA. The *cis*-9, *trans*-11 CLA isomer, in particular, has been identified as a potent anti-carcinogen (Ha et al., 1987; Lee et al., 2005).

Because dairy products represent a key component in the North American diet, there is interest in developing strategies to modify the fatty acid composition of bovine milk fat to minimize fatty acids that are of concern to human health while maximizing those that have exhibited significant health benefits.

Feeding flaxseed to dairy cattle is a potential strategy to increase milk fat content of omega-3 and CLA; however, feeding high levels of PUFA to cattle remains a challenge due to biohydrogenating microbial species within the rumen. Feeding high levels of PUFA may increase the risk for impaired fibre digestibility (NRC, 2001) as a consequence to the toxic effect UFA have on fibrolytic microorganisms in the rumen (Maia et al., 2006). As a protective strategy against dietary UFA, many ruminal microorganisms are capable of the hydrogenation of dietary UFA to a more saturated, and therefore less toxic, form (Jenkins, 2008). The intermediates of this process include CLA isomers such as *cis-9, trans-11* and *trans-10, cis-12* in addition to *trans-11* C18:1 while complete hydrogenation results in an accumulation of the SFA C18:0 (Harfoot and Hazelwood, 1997). Although the synthesis of the *cis-9, trans-11* CLA isomer in the rumen can be seen as beneficial due to the associated human health benefits, complete biohydrogenation of C18:3 n-3 from flaxseed would limit rumen outflow and mammary gland uptake of this nutrient. Additionally, accumulation of the CLA isomer *trans-10, cis-12* may have major economic implications for producers who are paid a premium for milk fat since this fatty acid has been associated with MFD (Peterson et al., 2002b).

Due to the challenges associated with feeding PUFA to dairy cattle, it becomes clear that flaxseed must be offered in a form that provides partial protection of dietary C18:3 n-3 from microbial biohydrogenation to enable increased rumen flow of C18:3 n-3 and *cis-9, trans-11* CLA. Both whole-flaxseed and extruded flaxseed have been shown to provide partial protection

of C18:3 n-3 from the rumen environment resulting in increased flow of C18:3 n-3 and CLA from the rumen as well as an increased milk fat content of these fatty acids (Chillard et al., 2009; Doreau et al., 2009; Oba et al., 2009; Sterk et al., 2012; Neveu et al., 2013). However, little data is currently available to directly compare whole-flaxseed supplements and extruded flaxseed supplements on rumen metabolism and milk fat composition. More recently, research has focused on the potential use of plant secondary compounds to mitigate the effects of ruminal biohydrogenation on dietary PUFA. *In vitro* trials have demonstrated the ability of condensed tannins to inhibit the final steps in the biohydrogenation pathway resulting in an accumulation of CLA isomers in ruminal fluid (Kronberg et al., 2007). However, *in vivo* experimental results are inconsistent and more work needs to be done to evaluate different feeding strategies that include condensed tannins on the implications for ruminal lipid metabolism and milk fat composition. Therefore, the objectives of this study were to compare the effects of extrusion versus whole flaxseed on ruminal fermentation, omasal flow of nutrients, milk composition and fatty acid profile and animal performance. Additionally, this study compared the effects of two extruded flaxseed products with varying levels of condensed tannins on the same parameters.

Results of this study suggest that whole-flaxseed and extruded flaxseed supplements can be safely included in the diets of dairy cattle at 11.4% DM without negatively impacting ruminal fermentation, ruminal digestibility of nutrients or animal performance. In the present study, milk fat content was lower when the average of RAW, LPR and LPF were compared to the CTL (P = 0.033); however the milk fat yield was unaffected. The reason for the lower milk fat content may be a dilution effect since there was a tendency for milk production to increase with the RAW, LPR and LPF-fed cows compared to the CTL. These are important findings for producers who are paid a premium for milk fat and for Canadian producers whose quota obligations are based

on milk fat yield. Furthermore, the results of this study suggest that feeding extruded flaxseed over whole-flaxseed may improve the feed efficiency of the cow which would be an economical benefit for producers. The differences in feed efficiency may be related to differences in total-tract digestibility between whole-flaxseed and extruded flaxseed; however more research is needed to confirm these speculations.

According to this study, whole-flaxseed was more effective at protecting dietary PUFA from the activity of biohydrogenatating microflora compared to extruded flaxseed. Results of this study found that the RAW treatment resulted in the highest omasal flow rates of C18:3 n-3 PUFA at 56.9 g/d compared to the14.0 g/d observed in the LPR treatment. However, the increased omasal flow rates did not result in higher milk fat content of C18:3 n-3 when comparing the RAW and LPR treatments. Other research has indicated that whole-flaxseed has poor total-tract digestibility (Petit, 2010) which could limit the transfer of PUFA into the milk. Results of this study found that the LPR treatment had higher flow rates of *cis-9, trans-11* CLA at 4.29 g/d compared to the 2.49 g/d reported for the RAW treatment. These findings further support the idea that intact oilseeds are more effective at protecting dietary PUFA from biohydrogenation since CLA is an intermediate of the biohydrogenation process. Future research should investigate the differences in total-tract digestibility between whole-flaxseed and extruded flaxseed supplements to determine if digestibility is the main factor limiting transfer of PUFA from the small intestine into the milk.

Results of this study found that increasing the levels of condensed tannins in an extruded flaxseed product did not prove advantageous for preventing biohydrogenation of dietary PUFA and increasing the levels of omega-3 PUFA and CLA in the milk. The LPF-fed cows did have higher flow rates of C18:3 n-3 ($P = 0.014$) and CLA ($P = 0.033$) compared to LPR; however the

most likely explanation for this increase is due to the higher dietary intake of C18:3 n-3 in the LPF-fed cow compared the LPR-fed cows ($P = 0.049$). When comparing the milk fat content of these fatty acids, no differences were observed. It is possible that the level of condensed tannins included in the LPF supplement were not high enough to elicit a response; however more research will be needed to determine if this assumption is correct. Furthermore, this study observed a loss of condensed tannins through extrusion processing and therefore, using extruded flaxseed products as a vector for condensed tannins may not be a practical dietary strategy. Future research should investigate the effects of feeding an extruded flaxseed supplement and condensed tannins from a separate dietary source on ruminal fat metabolism and milk fat composition.

Overall, feeding either a whole flaxseed supplement or an extruded flaxseed supplement increased bovine milk fat levels of both omega-3 PUFA and CLA compared to animals that were fed a control diet. Additionally, feeding flaxseed supplements resulted in a decrease in C12:0, C14:0 and C16:0 SFA. Therefore, results of this study show that an ideal milk fat with elevated levels of C18:3 n-3, CLA and lower levels of certain SFA is achievable through supplementation of a cow's diet with flaxseed. Future research should seek to expand on these findings through human trials. Determining whether or not the levels of omega-3 PUFA and CLA are enough to provide a health advantage to consumers over conventional information is crucial for the advancement of this area of research.

7.0 CONCLUSIONS

Results of this study provide evidence that feeding whole-flaxseed or extruded flaxseed to dairy cattle can effectively increase milk fat content of omega-3 and CLA fatty acids without negatively affecting rumen fermentation, omasal flow of nutrients and production performance. This study also provides evidence that whole-flaxseed is more effective than extruded flaxseed at protecting dietary C18:3 n-3 from ruminal biohydrogenation. However, feeding extruded flaxseed supplements is more effective at increased the milk fat content the transfer of C18:3 n-3 compared to whole-flaxseed. Furthermore, feeding extruded flaxseed is more effective at increasing *cis-9, trans-11* CLA in bovine milk. This research also indicates that the inclusion of faba beans as a source of condensed tannins in extruded flaxseed supplements may increase omasal flow of C18:3 n-3 and CLA from the rumen; however, no advantage was observed when comparing milk fat content of either C18:3 n-3 or CLA. Based on the information attained throughout this study, it can be concluded that feeding extruded flaxseed to dairy cattle is a superior dietary strategy, compared to feeding whole-flaxseed, for increasing bovine milk fat content of omega-3 PUFA and CLA while maintaining rumen fermentation and improving animal performance. Furthermore, increasing the concentration of condensed tannins in an extruded flaxseed to 1.17 mg/g of supplement did not prove advantageous at increasing healthful fatty acids in bovine milk, in the current study.

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APPENDIX A

Complete chemical composition of experimental flaxseed ingredients.

	Supplements[1]		
	RAW	LPR	LPF
Dry Matter %	90.68	93.50	93.58
Crude Protein, % DM	22.88	23.25	24.53
Soluble Protein, % of CP	58.80	45.99	45.98
ADF % of DM	9.23	8.83	9.80
ADICP, % of DM	0.90	0.81	0.85
NDF, % of DM	18.00	19.05	20.98
NDF Digestibility (30 hr), % DM	10.18	10.80	10.88
NDF Digestibility (30 hr), % NDF	60.63	56.68	50.80
NDFICP, % of DM	3.50	3.48	3.22
Lignin, % of DM	3.72	3.11	3.18
Crude Fat % DM	27.48	22.35	22.53
Sugar % DM	5.15	5.33	4.58
Starch % DM	16.30	18.23	18.60
Non Fibre CHO, % DM	28.25	34.80	31.00
Ash % DM	3.85	4.01	3.92
Calcium % DM	0.23	0.25	0.24
Phosphorus % DM	0.49	0.52	0.53
Magnesium % DM	0.31	0.31	0.31
Potassium % DM	0.88	0.99	0.94
Sulfur % DM	0.23	0.24	0.24
Sodium % DM	0.03	0.03	0.03
Chloride % DM	0.06	0.08	0.06
Iron, mg/kg	231.75	311.75	234.75
Manganese, mg/kg	39.00	37.00	34.00
Zinc, mg/kg	44.75	46.75	44.00
Copper, mg/kg	11.00	11.50	12.00

APPENDIX A (Cont'd)

Complete chemical composition of experimental flaxseed ingredients.

	Supplements[1]		
	RAW	LPR	LPF
TDN, % DM	116.85	109.10	108.60
NE_m, Mcal/kg[2]	1.36	1.26	1.26
NE_g, Mcal/kg[3]	0.99	0.91	0.90
NE_L, Mcal/kg[4]	2.45	2.31	2.29

[1]Supplement: RAW = non-extruded flaxseed and pea product (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant; O&T Farms Ltd., Regina, SK), LPR = extruded flaxseed and pea product (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant (Ethoxyquin; Santoquin®; Novus International, Inc.; St. Charles Missouri); linPRO-R ®; O&T Farms Ltd., Regina, SK), LPF = extruded flaxseed and high tannin faba bean (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant (Ethoxyquin; Santoquin®; Novus International, Inc.; St. Charles Missouri); O&T Farms Ltd., Regina, SK).

[2] NE_m: Net energy of maintenance. Calculated using the Ohio State Summative Equations (Weiss, 1998).

[3] NE_g: Net energy of gain. Calculated using the Ohio State Summative Equations (Weiss, 1998).

[4] NE_L: Net energy of lactation. Calculated using the Ohio State Summative Equations (Weiss, 1998).

APPENDIX B

Concentration of condensed tannins in experimental flaxseed products

Condensed tannins(mg/g) in different flaxseed supplements fed to lactating dairy cattle pre- and post-extrusion processing

	Flaxseed Supplement[1]					
	RAW		LPR		LPF	
	Pre-extrusion	Extruded	Pre-extrusion	Extruded	Pre-extrusion	Extruded
Condensed tannin, mg/g	n/d[2]	n/a[3]	n/d	n/d	6.87	1.17

[1]Supplement: RAW = non-extruded flaxseed and pea product (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant; O&T Farms Ltd., Regina, SK), LPR = extruded flaxseed and pea product (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant (Ethoxyquin; Santoquin®; Novus International, Inc.; St. Charles Missouri); linPRO-R ®; O&T Farms Ltd., Regina, SK), LPF = extruded flaxseed and high tannin faba bean (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant (Ethoxyquin; Santoquin®; Novus International, Inc.; St. Charles Missouri); O&T Farms Ltd., Regina, SK).

[2]n/d: not detected.

[3]n/a: not applicable.

APPENDIX C

Complete chemical composition of experimental diets.

	Diets[1]			
	CTL	RAW	LPR	LPF
Dry Matter %	91.34	91.42	91.74	91.75
Crude Protein, % DM	16.33	16.76	16.80	16.94
Soluble Protein, % of CP	31.64	36.89	35.43	35.43
ADF % of DM	19.52	19.64	19.59	19.70
ADICP, % of DM	1.71	1.68	1.67	1.68
NDF, % of DM	30.39	30.64	30.76	30.98
NDF Digestibility (30 hr), % DM	15.23	15.30	15.37	15.38
NDF Digestibility (30 hr), % NDF	53.51	51.84	53.12	52.45
NDFICP, % of DM	1.90	2.09	2.09	2.06
Lignin, % of DM	4.05	4.17	4.10	4.10
Crude Fat % DM	3.21	5.91	5.32	5.34
Sugar % DM	4.67	4.64	4.66	4.57
Starch % DM	28.71	25.71	25.93	25.97
Non Fiber CHO, % DM	41.60	38.83	39.58	39.14
Ash % DM	9.59	9.00	9.02	9.01
Calcium % DM	1.13	0.99	1.00	0.99
Phosphorus % DM	0.50	0.48	0.48	0.48
Magnesium % DM	0.34	0.32	0.32	0.32
Potassium % DM	1.73	1.72	1.73	1.73
Sulfur % DM	0.27	0.26	0.26	0.26
Sodium % DM	0.51	0.41	0.42	0.42
Chloride % DM	0.71	0.63	0.63	0.63
Iron, mg/kg	328.41	319.91	329.02	320.25
Manganese, mg/kg	64.03	58.91	58.68	58.34
Zinc, mg/kg	91.61	79.09	79.31	79.00
Copper, mg/kg	23.83	20.52	20.57	20.63

APPENDIX C (Cont'd)

Complete chemical composition of experimental diets.

	Diets[1]			
	CTL	RAW	LPR	LPF
TDN, % DM	68.70	73.30	72.41	72.36
Ne_m, Mcal/kg[2]	1.35	1.45	1.44	1.44
Ne_g, Mcal/kg[3]	0.72	0.78	0.73	0.77
NE_L, Mcal/kg[4]	0.45	0.50	0.46	0.49

[1]Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = diet including a extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant; linPRO-R ®); and LPF = diet including a extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant). All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK).

[2] NE_m: Net energy of maintenance. Calculated using the Ohio State Summative Equations (Weiss, 1998)

[3] NE_g: Net energy of gain. Calculated using the Ohio State Summative Equations (Weiss, 1998)

[4] NE_L: Net energy of lactation. Calculated using the Ohio State Summative Equations (Weiss, 1998)

APPENDIX D

Income over feed costs

	Diets[1]			
	CTL	RAW	LPR	LPF
Total feed cost $/cow/day	2.47	3.12	2.31	2.53
Milk value, $/cow/day[2]	28.00	28.02	28.18	27.45
Income over feed cost, $/cow/day	25.53	24.90	25.87	24.92

[1]Diets: CTL = control diet with no flaxseed supplement; RAW = diet including a non-extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant); LPR = diet including a extruded flaxseed and pea supplement (55% flaxseed, 36% peas, 8% alfalfa, 1% antioxidant; linPRO-R ®); and LPF = diet including a extruded flaxseed and high tannin faba bean supplement (55% flaxseed, 36% faba beans, 8% alfalfa, 1% antioxidant). All flaxseed supplements were manufactured by O&T Farms Ltd.(Regina, SK).

[2] Calculated based on SaskMilk marketing board 2015 component pricing.